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NEURAL AND ENDOCRINE FACTORS IN BODILY DEFENSE[1]

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It was in 1874 that Sir William Gull first described myxedema and connected it definitely with thyroid insufficiency. Ord confirmed this in 1878 and suggested the name. Murray successfully treated the first patient suffering from myxedema in 1891. He used a glycerine extract of the thyroid gland which he made himself.

Five years later, in the fall of 1896, there walked into my office a young woman whose face and hands were swollen; her eye-lids were almost closed, her voice was thick and her speech slow. She gave a history of progressive gain in weight and a gradually increasing apathy. I was impressed with the danger of approaching dissolution unless she could be quickly relieved, for I assumed the disorder to be of cardio-renal origin. I told her of the necessity of drastic action and prescribed the old-time remedies of purging and sweating. She replied that every doctor whom she had consulted had given the same treatment and that none of them had done her any good; and so she refused to be purged and sweated any more.

I then gave the matter a little more thought and recalled having seen such a case during my recent study in Europe, and remembered that it was said to be due to a failure of function on the part of the thyroid. I then knew that the case was not so urgent as I had assumed, and instead of purging and sweating I ordered desiccated thyroid. She started to improve at once and was soon able to resume her work as a teacher in the public schools. Shortly thereafter I lost contact with her. However, I had impressed upon her the importance of continuing the thyroid for a long time—probably during the remainder of her life. I neither saw her nor heard directly from her until 2 years ago, although indirectly I had heard that she was well and able to carry on her work. At the end of a public lecture given at Riverside, California, in 1934, she sought me out and told me how well she had become after starting thyroid medication. She said that she had continued its use for a few years and then found that she no longer needed to employ it.

This case is interesting from several standpoints. In the first place it was one of the early cases of myxedema successfully treated. Although the myx-

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edema was of a high grade it yielded readily to treatment, and the thyroid gland after a time was able to function sufficiently to maintain a physiologic balance and keep the patient in normal health for a period of 36 years, and possibly many more.

This was my introduction to endocrinology 20 years before our Organization was formed. The transformation of this patient from serious illness to health was so prompt that the picture frequently came into my mind during those early years of practice, although I did not know that this was to be my introduction to an important phase of physiologic medicine, which within a quarter of a century was to engage the minds of many of the ablest men of science.

My interest in endocrinology was next stimulated in 1908-9, soon after discovering the motor and trophic reflexes which are produced in skeletal structures by inflammation (tuberculosis) of the lung. I was forced to seek the explanation of these reflexes in the realm of visceral neurology, a subject which at that time had all but escaped the notice of investigators. I was finally able to establish the fact that the mediation of these reflexes takes place in the cervical portion of the spinal cord, particularly the 3rd, 4th and 5th segments. But this discovery demanded the investigation of the entire field not only of reflex action but of general reaction in visceral nerves; which again led to a study of the broad subject of physiologic control of body activity.

From study chiefly of the contributions of Langley and his associates, Gaskell, Cannon, Higier, and Müller, it was learned that the visceral nerves not only afford a system of control of activity in vegetative structures but that the afferent system of nerves from the viscera form a network through which activities in one viscus are correlated with activity in other viscera and with skeletal structures through efferent nerves belonging respectively to the visceral and somatic system; and that no individual viscus is independent in action. These studies had not progressed far when I learned of a second integrating and correlating mechanism, chemical in nature, which consists of the products of the endocrine glands.

It was further found that a stimulus going over a nerve to a viscus might produce its expected effect, no effect, or an effect opposite to the one usually assigned to it. To account for this we learned that nerve response may differ with different states of activity of an organ at the time of stimulation. Since organs are made up of aggregations of cells their activity is thrown back on the state of the cells, which in turn are dependent for their action upon substances which reach them through the body fluids which bathe them. This forced consideration of the facts which maintain physiologic equilibrium, the condition of homeostasis so beautifully described by Cannon, the *milieu intérieur* of Brown-Séquard, recently elaborated by Barcroft, with its hormones, electrolytes, vitamins, enzymes, lipoid substances such as lecithin and cholesterol, the products of normal metabolism, and the abnormal products which result from disease.

It became evident that the humors of the body were again returning to a place of primary importance in medicine, and that their investigation was

necessary to an understanding of physiologic function which, after all, is the basis of clinical medicine. The approach which seemed essential to the understanding of the body fluids and which seemed richest in promise was through investigation of the products of secretion of the endocrine glands, for these were assumed to influence and control so many functions.

This approach has now been justified because it has already shown that hormones not only exert an effect on cellular activity in various organs, but also, circulating in the fluid systems of the body, correlate and integrate visceral activity, supplementing the action of visceral nerves. We now know that hormones exert an influence upon fecundation, the maturation of the embryo and sexual development; that they are determining factors in the somatic and psychic spheres; that they influence metabolic activity, exert a control over the electrolytes and pH of the blood, and provide a chemical regulating and correlating mechanism for the body. Endocrine glands are activated by visceral nerves and are influenced by many products circulating in the body fluids other than hormones from other glands. So in the development of endocrinology scientists are forging one of the important links of physiologic medicine—the medicine which will in all probability dominate research and practice during the next quarter of a century.

While the relationship of endocrinology to the chemistry of the body is gradually unfolding, a study of its relationship to visceral neurology is lagging far more than it should. When our organization was founded, some of us who were interested in both subjects hoped that visceral neurology and endocrinology, the two correlating and integrating systems of the body, would be developed together; but like structural and functional pathology, it seems that they first had to be divorced so that they might have a happy reunion later; but reunion they will have.

To make endocrinological research of greatest importance to the clinician and to translate it into the most useful service to the patient, it must be intimately connected with all of the mechanism which have to do with physiologic control of the body. The many interrelationships between the various glands which are now being investigated, and the results of hypofunction and hyperfunction are of tremendous importance but fall far short of the full meaning of endocrinology to medicine.

The conception that the symptom-complex which we designate as a disease represents primarily the condition which is necessary for the organism to assume in order to protect itself against the morbific elements which play the etiologic rôle appeals to me as being most reasonable. With this conception, both inflammation which marks local reaction, and the general reaction of the body to toxic substances, become protective phenomena.

Substances which call forth reaction enter the body in two ways: *1)*, through the gastrointestinal tract, being subjected to natural digestion, in which case the readily diffusible products are absorbed and assimilated for the benefit of the organism and the useless ones rejected from the body; *2)*, through the walls of the gastrointestinal tract, having escaped natural digestion therein; and through other mucous membranes and through the skin. In case

of bacteria and protein substances in group *2)*, a different process of digestion and elaboration is necessary. If the substances are readily diffusible, they enter the cells directly, and are dealt with by a process similar to that used by unicellular organisms. Bacterial antigens are composed of a nucleoprotein and a carbohydrate-containing fraction. Sensitization of body cells, which is an exaggeration of their normal defense function, is produced only by the two fractions combined. The nucleoprotein is the fraction which is associated with the tuberculin reaction, while the carbohydrate-protein is the specific fraction that reacts with antibodies. Though both combined are necessary to produce sensitization, the nucleoprotein will call forth reaction after sensitization has been established. The soluble carbohydrate product is specific in nature and has a special action in dilating capillaries and acting upon smooth muscles. This substance differs with different diseases.

We conceive that every entrance of a foreign substance into the tissues calls forth a defensive mechanism on the part of the organism to deal with it. The mode of attack varies with the nature of the entrant and the tissues in which it finds itself. However, no matter what the nature of the foreign material and no matter where the reaction takes place, the defense phenomena represent normal, or an exaggeration of normal, cellular functions. In other words, defense is an exaggerated physiologic function in which there takes place a correlation and integration of the activities of the body which are useful in maintaining efficient body action while opposing the injurious substances which have found their way into the tissues. In spite of this assumed protective nature of the body's reaction it is not inconceivable that the reaction caused by morbific elements might cause such a concentration of physiologic effort for defense purposes that the normal physiologic equilibrium should fail to be maintained. This failure may be local or general; it may be transitory or continuous; it may be mild or serious. It may even prove to be so serious as to threaten or actually cause a stoppage of physiologic function and death.

That hormones have a part in aiding the organism in some way in carrying out parenteral digestion and in protecting the body from an injury that might result therefrom is suggested by clinical experience. We see evidence of this in the parenteral digestion of bacterial antigens and even more plainly in case of antigens responsible for the so-called allergic group of diseases in which the deleterious effects are often prevented and quickly relieved by adrenal hormones.

If we attempt to apply this idea to the solution of the riddle of specific defense or immunity to infectious diseases, assuming that protection is quickened and heightened physiologic action, then complete immunity is not only a rapid response of heightened reaction but a permanent ability of body cells to function quickly with heightened physiologic action; an ability so efficient at times as to make it impossible for certain microörganisms to grow and develop within the body, as is shown in case of such diseases as smallpox, typhoid fever, scarlet fever and diphtheria.

In diseases such as tuberculosis, pneumonia, steptococcal and staphylococcal infections, a heightened physiologic response is evident but not completely

protective. In some instances, of which tuberculosis is the best known example, protection appears both as a quickening and a heightening of the body's reaction whenever causative microörganisms come in intimate contact with the animal body after a primary infection has once been established.

The relationship of hormones to resistance to infections offers a fruitful field for study. The thyroid, adrenal and pituitary glands, particularly, have been suggested as being closely associated with resistance, and it is common clinical interpretation that hypergonadal and hypogonadal subjects resist infection with different degrees of efficiency. We have long used thyroid in the treatment of our tuberculous patients, seemingly with advantage to the patients. The observation that first caused us to use it was the dry skin which had been thought to be due to fever. We found, however, that it is present at times when fever cannot possibly be the cause, so we assumed that it was due to a deficiency in the action of the mechanism which controls the nutrition of the skin, and we administered thyroid as being the substance which seemed most apt to relieve this condition and were gratified not only in the effects on the skin but on the general stimulus which it seemed at times to give to nutrition and to the patient in fighting his infection.

More recently we have had experience with the products of the adrenal gland in treating certain infections. We have found that the duration and severity of colds have been lessened. We have had an unusual opportunity to observe this in certain children whom we have been treating for asthma. Whereas prior to treatment colds were frequent and usually followed by bronchitis and asthma, after treatment was established the colds lessened in number and severity, and the bronchitis and asthma gradually disappeared.

In tuberculosis we thought that there might be some relationship between the condition of the adrenals and the persistent tiredness which often accompanies but which more particularly follows the toxic stage of the disease. Francis M. Pottenger, Jr., made this a study and found that he was able not only to relieve the tiredness but also to produce unmistakable general improvement in patients by feeding raw adrenal glands or potent extracts of the cortical portion of the gland. He was also able by the cortical hormone to protect about 30 per cent of three different series of guinea pigs against infection with the tubercle bacillus, and to cause a more favorable course in the disease of those infected. Another observation that is worthy of consideration is that he has seen the reaction of hypersensitivity to tuberculin, also the sensitivity to antigens in asthma disappear in children whom he has treated with adrenal products.

Of the many patients so far treated for asthma, improvement in the general physical state was practically invariable. We can hardly suppose that these effects are produced because of any specific action that the hormones might possess but rather by the general restoration and maintenance of the physiologic equilibrium. However, the fact that by the use of adrenal hormones we have been able to prevent in animals infection with tubercle bacilli, to cause children who reacted positively to the tuberculin test to cease to react, and to eliminate the hypersensitivity of asthmatics to certain proteins would indicate

that they confer upon the animal an unusual degree of competency in opposing proteins which require parenteral digestion for their destruction. May we not interpret this disappearance of the tuberculin reaction and this loss of sensitiveness to allergens during the treatment of asthmatic children with adrenal hormones as evidence that a refractoriness to antigens has been produced, such as would be found in a more complete immunity. The time which has elapsed so far is not sufficient to warrant a too close interpretation, nor has it been sufficient to permit of more complete investigation. Nevertheless, this experience suggests that a better understanding of the protection of the body against bacteria and other sensitizing substances which require parental digestion for their destruction may come through the study of endocrinology.

Our development of endocrinology has followed much the same course as the development of other phases of medicine. First the disintegrating method of studying individual glands and their products had to be pursued; but this must now give way to a process of integration, of fitting our knowledge into the whole picture of physiology.

In the development of the endocrine system we are furthering knowledge of both normal and pathologic physiology, and adding to the understanding of man in health and disease.

EMPHASIS OF THE GROWTH EFFECT OF PROLACTIN ON THE CROP GLAND OF THE PIGEON BY ARREST OF MITOSES WITH COLCHICINE

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The crop gland of the adult pigeon undergoes marked cyclic changes, which Champy and Colle connected with modifications in the gonads during the reproductive cycle (1, 2). Within the 10 days preceding the hatching of the young, the crop wall thickens approximately from 0.04 mm. to 4 mm., and many mitoses in the epithelium testify to this considerable growth (3, 4, 5, 6).

Riddle, Bates and Dykshorn attributed the proliferation of the crop gland to a release of lactogenic hormone (prolactin) by the hypophysis, and reproduced it by injection of this hormone into adult or immature pigeons (7, 8). In a crop gland stimulated in this way two kinds of changes occur; namely, multiplication of the cells and deposition of fat globules in them. Mitoses found in the sections give evidence of the cellular multiplication. However, their number cannot be used as a test of the growth activity of the tissue, since observations on living tissues show short periods of extensive mitotic activity interspersed with long periods of general cell rest.[1] Therefore, when the animal is killed, pulses of cell division may happen to be caught, but more frequently they will be missed.

Dustin has shown that an alkaloid, colchicine, if injected into an animal will arrest the cells in division (10). This substance which, according to Ludford (11) and Brues (12), does not increase the rate of mitosis, prevents the divisions from going past the metaphase stage. Thus the cells, which divide during the period of the activity of colchicine will be accumulated in easily recognizable condition, so that colchicine acts as a reagent to manifest the mitotic activity of a tissue in given circumstances (10 to 16). Allen, Smith and Gardner (17, 18) used colchicine to study the growth effect of theelin on genital tissues in the sprayed mouse. The results showed clearly the dominance of this hormone over hyperplasia in these organs.

The present article describes an attempt to accentuate, by means of colchicine, the evidence of growth response of the crop gland to prolactin.

METHOD

The responses of the crop gland to systemic and local injections of prolactin and to implantations of hypophyses of rats were examined in 28

[1] This fact has been shown by observations on living tissues, chiefly by the motion pictures of tissues cultures (W. H. Lewis) or developing embryos (Amblystoma: L. S. Stone and T. C. Kramer; Siamese Fighting Fish: C. P. Leblond and T. C. Kramer). However, Brues and Marble (9) conclude from their researches on regenerating liver that "no great number of mitoses begin or end simultaneously."

pigeons, 18 of which were white Carneaux, 6 to 8 weeks old, the others being adult ordinary pigeons. For the study of the systemic response to prolactin, 11 animals were treated as follows. They received a single subcutaneous injection of 40 bird units of the hormone in the leg.[2] Approximately 24 hours later they received various amounts of colchicine[3] and were killed 9 to 11 hours after the colchicine injection, that is to say, about 36 hours after the injection of prolactin. Controls received colchicine alone (7 animals) or prolactin alone (2 animals). For the study of the local reaction, the injections of prolactin or the implantation of hypophyses were performed over the right crop gland.

	No. of Animals	Amount of Colchicine mg.	Prolactin B.U.	Av. gross development	No. of mitoses for 2000 cells			Average mitotic index %
					Average	Smallest no.	Greatest no.	
Colchicine controls	2	0.10–0.25	—	0	12	9	15	<1
	6	0.40–0.50	—	0	46	8	173	2
Prolactin controls	2	—	40	3–4	15	9	21	<1
Prolactin-Colchicine	6	0.10–0.35	40	2–4	27	11	48	1
	5	0.50	40	2–4	534	210	1075	26

In all cases a rough grading from 1 to 4 estimated the extent of the gross development of the crop glands. Marked individual variations were noted in the responses to prolactin. The crop glands were slightly stretched and fixed in Helly's fluid or occasionally in salted formalin. They were stained with hematoxylin and eosin.

The percentage of nuclei undergoing motosis was determined by enumeration of both dividing and resting nuclei. This *'mitotic index'* was determined by enumeration of 2000 cells, counted in one of every 5 fields. As the mitoses predominate along the basal membrane of the epithelium, a true ratio requires that the cells be counted from the surface to the basal membrane. It may be noted that such an enumeration was performed in all the animals, but it seems unnecessary when there is obviously no increase in the number of mitoses; then the mitotic index is 1 per cent or less.

RESULTS

If colchicine alone was injected into an adult or immature pigeon, it had little effect on the crop gland (fig. 1, *A, A'*). Only a few mitoses were arrested, corresponding probably to the cells which would have normally completed division during the time of action of colchicine. With only one

[2] The prolactin was kindly furnished by E. R. Squibb & Sons through the courtesy of Dr. Anderson. The method of preparation and unitage is that described by Riddle, Bates and Dykshorn (19).

[3] The colchicine was prepared by Mallinckrodt (Eimer and Amend, New York); 1 mg. was dissolved in 1 cc. of distilled water.

exception,[4] the mitotic index was equal or inferior to 2 per cent in all individual cases (table).

In a second group of animals, the injection of prolactin alone induced the well known proliferation of the crop gland (fig. 1, *B, B'*). Mitoses were frequent, chiefly along the basal membrane and were observed in all

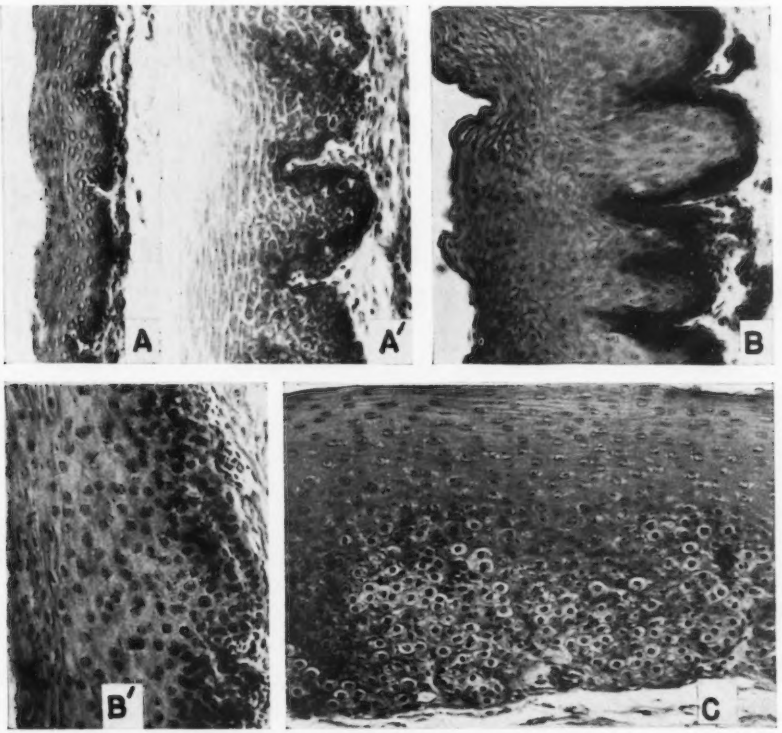

Fig. 1. Epithelium of the crop gland. *A, A'*, Resting crops after colchicine. A few mitoses are visible. *B, B'*, Stimulation with prolactin, no colchicine. No mitoses are visible here. *C*, Stimulation with prolactin and treatment with colchicine. 23 per cent of the cells are in mitosis.
All fixed in Helly's fluid and stained with hematoxylin, eosin, except A' which is fixed in formalin and stained with toluidin blue. × 192.

stages. However, the mitotic index was less than 1 per cent. In order to determine the amount of colchicine necessary to arrest the mitoses, 11 animals treated with prolactin received various amounts of colchicine. A large number of mitoses was observed in animals receiving over 0.35 mg. of colchicine (table).

In the animals given 0.50 mg. of colchicine after prolactin treatment a considerable number of mitoses was observed (fig. 1, *C*). Almost all the cells along the basal membrane of the crop gland epithelium were dividing.

[4] In 1 of the 8 animals given colchicine alone, 173 mitoses per 2000 cells (8.6%) were found in an immature male Carneau. This discrepancy could not be explained.

An average of 26 per cent of the cells were in mitosis.[5] In one case (fig. 2), almost all the cells throughout the epithelium were dividing, with the exception of a superficial layer of flattened senescent cells. In a square millimeter of epithelium, 16,917 mitoses were counted (section 10 μ thick). The ratio of dividing nuclei to the total number of nuclei was 53 per cent.

The average number of mitoses was 37 times as great as in the controls injected with prolactin alone, although the thickness of the epithelium was approximately the same. According to Ludford (11) and Brues (19) these results are a true picture of the number of cells, that in normal circumstances

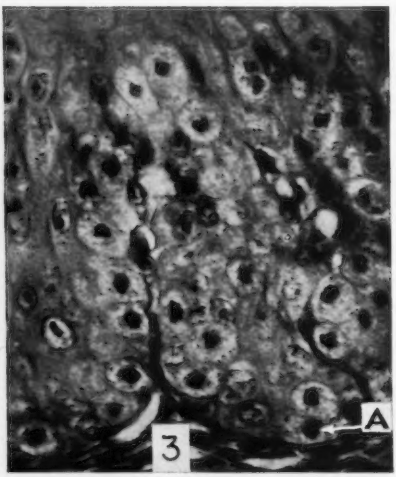

Fig. 2. Colchicine treatment after stimulation by prolactin. 53 per cent of the cells are dividing. × 100.
Fig. 3. Higher power view of the mitoses. They predominate along the basal membrane of the epithelium. All mitoses are arrested in metaphase with clumping of the chromosomes, except for one anaphase (A). × 440.

would have completed division during the 10 hours of the action of colchicine. This implies that during 10 hours, there are 37 times as many dividing cells as are observed at any moment in the prolactin treated animals. If one supposes that the occurrence of mitoses is regular, as it is in the regenerating liver (9), these observations indicate that the duration of mitoses in the growing crop is 16 minutes.

Examination under high powers of the microscope showed the dividing cells crowded along the complicated folds of the basal membrane and bulging often into the connective tissue, either individually or in groups (fig. 3).

Rare prophase stages are observed, characterized as normally by a slight increase in size of the nucleus and the appearance of a dark intra-nuclear recticulum. Most of the mitoses are in the metaphase stages. When at this stage, the columnar cells of the basal membrane or the flattened polygonal cells in the outer layers of the epithelium become very swollen and therefore spherical. The cytoplasm, which is rather basophilic in the cells of the basal

[5] The 5 animals in this group were males, 3 immature and 2 hibernating adults.

membrane and eosinophilic in the others, loses its affinity for dyes and appears very transparent. The chromatin nuclear material is densely packed in the centers of the cells, possibly because of a shortening of the chromosomes, such as observed by Ludford in tissue cultures (11). The polar views of mitosis show a tendence to a circular arrangement of indistinct chromosomes at the equator of the cells. The spindle and centrosomes are usually not distinct; they might be absent (11). Rarely anaphases and telophases are observed. The spindle is then usually visible. Occasional mitoses have been found in the connective tissue.

In the last series of experiments the injection of colchicine was used to accentuate the growth produced in local tests of prolactin as proposed by Lyons (20). Three animals received single intracutaneous (21) injections of 10, 5, and 5 B.U. of prolactin, and were killed 9, 20, and 24 hours later. Marked reactions appeared at the site of injections extending somewhat to the other crop gland. The occurrence of a very large number of mitoses as soon as 9 hours after injection enables one to determine the presence of prolactin in a very short time.[6]

In 2 animals, implants of rat pituitaries under the skin of the crop gland stimulated mitotic division, visible 24 and 48 hours after the implantation.

The use of the colchicine method for bio-assay of prolactin seems practicable. The ratio of dividing cells to the total number of cells might be used as an index of the growth of the crop gland.

SUMMARY

After injection of colchicine which arrests dividing cells in the metaphase stage for long periods, the evidence of growth (mitogenetic) produced by prolactin in the crop gland of the pigeon was accentuated by the finding of large numbers of mitoses in the epithelium. The effective dose of colchicine for a 400 to 500 gm. pigeon is 0.5 mg.

The percentage of nuclei undergoing mitosis (*mitotic index*) in a prolactin-treated pigeon is approximately 1 per cent, whereas after injection of colchicine in such an animal, it increases to 26 per cent. The growth produced by local injections of prolactin under the skin of the crop gland could be detected with colchicine as soon as 9 hours after injection, by the appearance of numerous mitoses.

By the same method, the growth determined by implantation of hypophyses of rats was examined.

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[6] The use of the freezing microtome after a short formalin fixation (20 minutes) enables one to verify the presence of mitoses in a short time.

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THE EFFECTS OF HYPOPHYSEAL IMPLANTS FROM NORMAL MATURE GUINEA PIGS ON THE SEX ORGANS OF IMMATURE GUINEA PIGS

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The present report concerns the effects of hypophyseal implants from normal guinea pigs at different stages of the estrous cycle. Since a survey of the literature showed no complete report of the gonadotropic activity of the guinea-pig hypophysis, this work was a necessary prerequisite to the study of the effects of hypophyses from guinea pigs whose ovaries had been irradiated.

Smith and Engle (1) have reported, in an abstract, that the responses to implants of guinea pig pituitaries varied with the stage of the estrous cycle of the donors; there was but slight ovarian response (in immature rats and mice) to an implant from guinea pigs whose vaginae were open, but the response was satisfactory when the donors were in diestrus, from the 5th to the 14th day after stage I of estrus.

METHODS

All animals used were laboratory stock of known history. Estrous records of all prospective donors were kept; the stages of estrus were determined by the vaginal smear technique of Stockard and Papanicolaou (2). Most of the donors were virgin females weighing between 400 and 700 gm. Some smaller and larger ones were included also, as well as a few multiparas, primiparas and immature females, and a few males. The recipients were immature female guinea pigs weighing between 190 and 210 gm.; these were about 4 weeks old.

For the experiments, the donors were killed quickly with chloroform, the hypophyses were removed, weighed rapidly on an analytical balance, and implanted into a subcutaneous pocket on the abdomen of the recipients. Each of 50 recipients received but a single hypophysis as an implant, so that a comparison could be made with the effects of a single hypophysis from animals with irradiated ovaries. Twelve recipients received 2 hypophyses at one time, and 6 received 4 at one time, from donors in the same stage of the estrous cycle. The recipients were killed 6, 7 or 8 days later. Organs for study were fixed in Zenker's fluid, paraffin sections were made, and stained with hematoxylin and eosin. Organs for study from the donors were treated similarly.

OVARIES AND ACCESSORY SEX-ORGANS IN IMMATURE CONTROLS

The ovaries of guinea pigs weighing about 200 gm. are small, but contain abundant follicles of all sizes up to medium-sized or somewhat larger. No mature follicles are present. The follicles degenerate by filling in with connective tissue. The

[1] Much of the technical part of this work (preparation and staining of sections) was done at the Marine Biological Laboratory, Woods Hole, Massachusetts.

abundant primordial follicles are mostly in a resting condition, and new primordial follicles are forming from the germinal epithelium by amitosis.

The uterus is very small in diameter. The lining epithelium is low columnar; some mitotic figures are present. The glands are small, without mitotic activity. The tunica propria is compact. At times there is slight proliferative activity in the tunica propria immedately under the epithelium. There is no proliferation in the smooth muscle.

The vagina is small. The epithelium bordering the lumen is a stratified columnar type; the cells of the outermost row are cylindrical, mucous-secreting cells with a central nucleus; these rest on 1 or 2 rows of small, flattened or cuboidal cells, some of which are dividing by mitosis. The vaginal lumen is very convoluted, and has not attained the characteristic canalization found in the sexually mature animal. The vaginal orifice is closed over by a membrane, which does not rupture until the first estrus.

GENERAL EFFECTS OF HYPOPHYSEAL IMPLANTS

Ovaries of recipients. The ovaries, when affected by the implant, were stimulated to follicular growth. Follicles grew rapidly to a larger size, but the largest follices found were, at the most, only about two-thirds the size of the mature follicles found in sexually mature ovaries. These follicles always degenerated without further growth and without formation of luteal tissue. In no instance was there any formation of luteal tissue, with the single exception of the ovaries stimulated by double male hypophyseal implants.

Usually the existing follicles grew to a larger size. At times, only primordial follicles were stimulated to rapid division, so that increased numbers of small follicles were formed. In some instances, both processes occurred simultaneously. Following quadruple implants, the growth effect on the follicles was somewhat accentuated, in that more follicles grew to a large size. Larger growth of individual follicles did not occur; the largest ones were still only two-thirds the size of typical mature ones.

Vagina. A typical estrous condition occurred frequently in the vaginae. When it occurred following a single implant, the vaginal membrane ruptured 4 or 5 days after implantation. Following a double or quadruple implant, the vaginal membrane ruptured sooner, usually in 2½ to 3 days. In some instances, the estrous reaction was weak, that is, the epithelial layers were fewer, the various stages of estrus were not so sharply defined, and the vagina was smaller grossly.

Uterus. Activity in the uterus paralleled that in the vagina, to some extent, but the degree of activity was somewhat variable and not always as pronounced. The changes occurring in the uterus were those associated with estrus and postestrus. In addition, mitotic proliferation was observed in the smooth muscle cells, following certain implants.[2]

Mammary gland. The blood vessels in the region of the gland were dilated, macroscopically, in most instances where an active vaginal estrus occurred. Microscopically a few enlarged ducts were seen, sometimes a few acini. The effects were somewhat variable. Following double and quadruple implants,

[2] Mitotic figures were counted in 3 or 4 sections of the same uterus; the number per section was constant. The average was recorded.

more marked proliferation of ducts and acini occurred in all instances where an active vaginal estrus had occurred. Loeb (3) reported similar development after male guinea pig implants.

Thyroid and adrenal glands. There were no changes in these organs, after either a single or double implant. Loeb (3) found no significant changes with as many as 4 male implants daily.

Effects of age, weight and state of sexual maturity of the donors. For any given period of the estrous cycle of the donors, the reactions in the recipients were generally similar regardless of the weights of the donors, within the limits used, or of the weights of the hypophyses. Variations in the weights of single hypophyses were small (average weight 13.5 mg.; maximum 16.5, minimum 10.1 mg.).

For any given period of the estrous cycle of the donors, the reactions in the recipient were generally similar regardless of whether the donor was a virgin, a primipara, or a multipara. Single hypophyses of sexually immature donors (285 to 330 gm.) were without effect on the recipients, as was also half a hypophysis from a sexually mature animal.

EFFECTS OF SINGLE AND DOUBLE IMPLANTS FROM FEMALE DONORS AT DIFFERENT STAGES OF THE ESTROUS CYCLE

Donors in Stages I to IV of Estrus, or Immediately Following Estrus (4 Days after Stage I)[3]

Ovaries of 6 recipients. Single and double implants had only slight or no follicle-stimulating effect. Ovaries remained small; follicles were limited to small and medium sized ones; only occasionally was a larger one present. Primordial follicles were chiefly in a resting condition. The germinal epithelium was no more active than in control animals.

Vagina. Single implants had no effects. Double implants were ineffective, except in one instance, where a weak estrous reaction had taken place, but the vaginal membrane had not ruptured.

Uterus. All uteri were small and inactive, with the exception of one where weak estrus had occurred in the vagina; here the tunica propria was proliferating slightly. There was no proliferation in the smooth muscle.

Donors 5 to 6 Days after Stage I of Estrus

Ovaries of 10 recipients. Both single or double implants had but slight follicle-stimulating effect. The ovaries were similar to those after estrous implants. Primordial follicles were resting in most instances.

Vagina. Even though the ovaries showed no decided follicle-stimulating effects, the vaginal membranes of all animals ruptured on the 4th or 5th day following the single implants, or on the 2nd or 3rd day following the double implants, and all had had a vaginal estrus. Grossly, the vaginae were considerably larger than those of control animals, except in 2 in which the estrous reaction was weak.

[3] It should be recalled that the average cycle length of the normal guinea pig is 16 days, during most of which time the vaginal orifice is covered by a membrane. This membrane ruptures at estrus, and remains ruptured for about 4 days.

Uterus. Grossly, all uteri were more enlarged than those of controls except the 2 accompanying the small vaginae. Some estrous activity was present in all. Proliferation in the tunica propria was evident, though not always extensive. The lining epithelium was proliferating. In some instances this epithelial proliferation extended into the glands for a short distance; this was more marked following double implants.

Little or no proliferation of smooth muscle occurred (0 to 8 mitoses per section); in 1 instance, 18 mitotic figures were seen; no explanation was available for this increase.

Donors 7 to 10 Days after Stage I of Estrus

Ovaries of 16 recipients. Following implants from donors 7 to 10 days after estrus, follicular stimulation was generally greater than in previous

TABLE 1. MITOSES IN UTERINE MUSCULATURE.

Stage of estrous cycle of donors	Implant	Av. no. mitoses per section of uterus (recipients)	Minimum and maximum mitoses
I–IV of estrus	Single or double	0	
	Quadruple	0	
5–6 days after estrus	Single or double	5	0– 8
	Quadruple	2	
7–10 days after estrus	Single or double	9	0–17 (1 case 23)
	Quadruple	52	
10–11 days after estrus	Quadruple	14	10–36 (1 case 61)
10–15 days after estrus	Single or double	28	
12–14 days after estrus	Quadruple	60	
Control, immature; wt. 200 gm.		2	0– 8 (5 cases)
Control, immature; wt. 250 gm.		0	(1 case)
Control, immature; wt. 215 and 275 gm. spontaneous cornification		4	0– 9 (2 cases)
Control, sexually mature, proestrus		0.6	0– 1 (3 cases)
Control, sexually mature, estrus		11	4–25 (4 cases)

series; more medium-large and large follicles were present, so that the ovaries as a whole were larger. Primordial follicles were dividing by mitosis and small follicles were forming, in half the cases of this entire group, but were resting in other instances; this did not seem to be correlated with the number of large follicles present.

Vagina. In spite of this increased follicular growth in the ovaries, the vaginae remained small, closed and inactive. In only 2 (of 6 single implant cases) of the 10-after-estrus group, was there enlargement of the vaginae, rupture of the membrane, and a typical estrous reaction. The ovaries of these animals were no different than those of others in the same group, in fact, no large follicles were present, merely many medium-large ones. Two double-implant cases likewise had enlarged vaginae in estrus with similar ovaries. Only 1 of the 7-after-estrus group, showed some reaction—proestrous activity in the vagina. Thus, all the vaginae of this series were inactive except a very few at either extreme of the group.

Uterus. All the uteri were small and inactive, except in the instances where the vaginae were in estrus. These uteri were somewhat enlarged grossly, with activity in the tunica propria, in the epithelium along the lumen, and in the epithelium of the terminal portions of the glands. Mitoses in the smooth muscles were infrequent; in some cases, a few were found; the greatest number, 23, was found following a double implant.

Donors 11 to 15 Days after Stage I of Estrus, Including Some in Proestrus

Ovaries of 13 recipients. The maximum follicle-stimulating effect was attained in this group. As a whole, the ovaries were large and very vascular, full of growing follicles, with many medium-large and as many as 5 large follicles. The germinal epithelium was proliferating actively, so that young primordial follicles, alone or in cell nests, were numerous. However, in 2 instances, both ovaries were small, with only 1 medium-large or 1 large follicle in a pair.

Vagina. In all animals, the vaginal membranes ruptured within 4 days following the implant, sooner in some, or within 2½ days following double implants. The vaginae were greatly enlarged, all had the characteristic estrous activity. In several, particularly those whose membranes ruptured sooner, there was some prolongation of the cornification stage of estrus. The 2 animals with the small ovaries had smaller vaginae, but estrus had been typical, though weak in one.

Uterus. In all, except the 2 associated with the smaller vaginae, the stimulation was much more advanced than in any described so far. The uteri were large grossly. The peripheral blood vessels were greatly dilated, the capillaries in the tunica propria were enlarged. The entire tunica propria was active; it was marked off sharply from the compact muscularis because of its loose structure. The lining epithelium was high columnar, mitotic figures were numerous; in places it was stretched into a flattened layer, due to the increased tissue in the tunica propria. The glands were enlarged, tortuous and prominent, with numerous mitotic figures at their terminal ends and sometimes at their proximal ends. In some instances, a postestrous condition was present—taller, more lightly staining lining epithelial cells with few mitotic figures, and mitotic proliferation in the proximal portions of the glands. The smooth muscle was proliferating actively (28 mitoses per section).

QUADRUPLE IMPLANTS FROM FEMALE DONORS

Four hypophyses taken from animals in the same stage of the estrous cycle, and implanted simultaneously caused ovarian stimulation, vaginal estrus, and uterine stimulation in all instances, but in varying degrees depending again on the stage of the estrous cycle of the donors. The degree of follicular stimulation was somewhat greater than after single or double implants in that more follicles grew rapidly, but again, the largest follicles were merely large and not mature. No ovulation occurred and no corpora lutea were formed.

The vaginal membranes ruptured in 2½ to 3 days in all instances except

following implants from donors in estrus; here it was delayed until the 4th day. These latter recipients were only in stage II or III of vaginal estrus when the other series showed complete repair (7 days after implantation). The 12 to 14-after-estrus implants again produced the maximum stimulation; the recipients showed some prolonged cornification in the vagina.

Uterine activity was present in all, but most intense in the 7 to 14-after-estrus group. The degree of mitotic activity in the smooth muscle paralleled that following single or double implants, but many more mitotic figures were present in correspondingly active cases. There was no mitotic activity following estrous or postestrous implants, but great activity following a 7 to 8-after-estrus and a 12 to 14-after-estrus implant; that following a 10 to 11-after-estrus implant was not so great, but instead, these tissues were greatly hypertrophied.

EFFECTS OF IMPLANTS FROM MALE DONORS

Following a single implant, the ovaries, uteri and vaginae of recipients were similar to those after the most active female implants; in addition, follicles grew to a larger size, so that 1 pair of ovaries contained at least 2 large follicles and 1 or 2 almost mature follicles. The latter degenerated without formation of corpora lutea. Smooth muscle proliferation in the uterus corresponded to that of the most active female implants (average, 30 mitoses per section).

Following double male implants, the ovarian stimulation was carried still further in that ovulation occurred in the 1 or 2 fully mature follicles, followed by formation of typical corpora lutea. The vaginal membranes ruptured 1.5 days after implantation; estrus had been completed on the 6th day. The uteri showed the typical postestrous structure—tall, lightly staining lining epithelial cells with few mitoses, enlarged convoluted glands with abundant mitoses in the epithelium of the proximal portions. Mitoses in the muscle were numerous (16) and, in addition, muscle cells were hypertrophied.

Nelson (4) has shown recently that the gonadotropic effect of male guinea-pig pituitaries is 50 per cent greater than that of females.

DISCUSSION

The results obtained in the present series of experiments can probably be explained by cyclic fluctuations in the follicle-stimulating substance present in the pituitary. If a luteinizing substance is present in the guinea-pig pituitary, it is not abundant, since none of the changes associated with it [generalized luteinization of the theca cells (5)] occurred after any of these implants; Loeb and his workers showed also that the growth-promoting substance (that is, the follicle-stimulating substance) was predominant in male guinea-pig pituitaries (6, 7) and that many implants daily were necessary before the luteinizing effect appeared in the ovaries of the recipients (7).

Probably some follicle-stimulating substance is always present in the pituitary; it is at its minimum during estrus, increases slowly during diestrus, then rises rapidly towards the end of diestrus, and during proestrus. Such an interpretation agrees with the cyclic variations in the cell types of the pituitary of the guinea pig (8). The basophils, which have been shown to be associated

with the follicle-stimulating substance, are most numerous and most granular in proestrus, indicating maximum secretory activity; they undergo degranulation and reduction in relative percentage during estrus, and remain at a low level during the lutein phase (8). This would explain the absence of follicular stimulation in the ovaries of the recipients during estrus, with increasing stimulation towards the end of diestrus and a maximum stimulation during proestrus. It is known that pituitary implants act on the vagina and uterus through the ovaries by producing follicular stimulation and consequently theelin (9). Probably more theelin is required for vaginal stimulation than for follicular stimulation; therefore, during early diestrus, some ovarians stimulation occurs, but no vaginal or uterine stimulation; later in diestrus and in proestrus, both vaginal and uterine stimulation occur. Other workers have shown that theelin will produce mitotic division of smooth muscle in the uterus. Here, smooth muscle was stimulated markedly after the most active implants—those from donors in later diestrus or proestrus; stimulation following other implants was comparable to that seen during a normal estrous period in sexually mature animals.

It is difficult to explain the occurrence of vaginal estrus and some uterine stimulation following the 5 to 6-day-after-estrus implants, yet it occurred in every instance. The study of the cyclic variations of the eosinophiles (8) may aid in the explanation. It is thought that the eosinophiles are associated with the inhibiting substance (or the luteinizing substance). They are most numerous and most granular in early estrus (8), perhaps exerting an inhibitory influence on any small amount of follicle-stimulating substance which may be present. Degranulation and reduction in relative percentage of eosinophiles occur in late estrus and more so in metestrus (8), indicating perhaps that the inhibitory substance is at its minimum, thereby allowing the small amount of follicle-stimulating substance present to exert its effects. Both eosinophiles and basophils remain at a low level during diestrus (8); the basophilic substance is probably predominant, sufficient to allow some ovarian but no vaginal stimulation in recipients. The basophilic substance becomes increasingly predominant in proestrus, obscuring any inhibitory substance which might be present.

Quadruple implants show that there is some basophilic substance present all the time, even during estrus, for here, ovarian stimulation occurred in the recipient, vaginal and uterine stimulation also, following estrous implants, but it appeared more slowly than in any of the other quadruple series.

It should be pointed out that the reaction in the vaginae and uteri did not always parallel the amount of theelin, assuming that the theelin is contained in the follicles, since in some few instances characteristic vaginal and some uterine changes occurred when the ovaries were small and follicles were small; in such instances, mitoses in the uterine muscle were fewer. Similar findings were reported by Wallen-Lawrence (10) in some of her experiments on rats (using sheep pituitary extracts), where estrous and postestrous changes occurred in the vagina and uterus, even though there was no significant increase in ovarian weight.

The occurrence of vaginal estrous changes in the guinea pig in the presence

of large but not mature follicles has been observed in normal animals (3, 11) and experimentally when most follicles have been destroyed and only one develops to a large size (12). In the present experiments also, vaginal and uterine estrous changes were produced, though mature follicles were not present in any instance except following double male pituitary implants. It is interesting to note again, that apparently more theelin is required for uterine estrous reactions than for vaginal estrous reactions, a finding pointed out by Marrian and Parkes in rats (13) and Schmidt in guinea pigs (12).

SUMMARY

Hypophyses from normal sexually-mature female guinea pigs, removed at different times during the estrous cycle, were implanted into immature female guinea pigs (single or double implants). Hypophyses from donors in estrus were ineffective. Hypophyses from donors 5 to 6 days after estrus caused little or no follicular stimulation, but vaginal membranes ruptured, typical vaginal estrus and some uterine proliferation occurred. Hypophyses from donors 7 to 10 days after estrus caused growth of follicles to medium or larger follicles, but vaginae remained closed and in a resting condition, the uteri were small and inactive. Hypophyses from donors 11 to 15 days after estrus caused the maximum follicle-stimulating effect—large ovaries, many medium and larger follicles, and proliferation of the germinal epithelium. Vaginae were greatly enlarged, the membranes ruptured, estrus occurred. Uteri were large with marked estrous or postestrous activity, and smooth muscle cells were proliferating. In no instance were follicles in the recipients fully mature; no ovulation occurred and no corpora lutea were formed, nor were the uteri and vaginae as large as in sexually mature animals in estrus.

Following quadruple implants, growth of follicles, vaginal estrus, and uterine changes occurred after all implants. Estrous reactions appeared last in recipients receiving estrous implants, and were most intense after proestrous implants, as was also smooth muscle proliferation in the uterus.

Hypophyses from male donors were still more effective, producing mature follicles, ovulation and formation of corpura lutea in the recipients, and marked estrous activity in the uterus and vagina.

The results can probably be explained by the fluctuation in the amount of follicle-stimulating substance present in the pituitary, influenced perhaps by a slight inhibitory effect of the substance produced by the eosinophils.

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THE EFFECTS OF HYPOPHYSEAL IMPLANTS FROM GUINEA PIGS WITH IRRADIATED OVARIES ON THE SEX ORGANS OF IMMATURE GUINEA PIGS

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Changes in the sex organs of guinea pigs following irradiation with a minimum sterilization dose of x-rays, the effects on the estrous cycle and on subsequent pregnancies have been described (1, 2). Although the structure of most of these irradiated ovaries was altered greatly, there was reason to assume that a part of their normal function was retained, namely, the secretion of theelin. This assumption was tested by a study of the urinary theelin excretion; since theelin was found present, the amount excreted was compared with that excreted by normal animals (3) and at the same time, the effects of hypophyseal implants from such irradiated animals were compared with the effects of hypophyseal implants from normal animals.

METHODS

The region over the ovaries of sexually mature guinea pigs was irradiated with a total of 2160 R, given as 6 half-hour treatments within a week (2). The animals used for the implantation experiments were the same ones used for a study of the excretion of theelin in the urine (3); they were killed from 5 to 10 months after irradiation. Three, which had been irradiated about 2 years previously, were also used.

Most animals were killed immediately after a urine collection. The hypophyses were removed rapidly, weighed on an analytical balance and implanted into subcutaneous pockets on the abdomens of immature guinea pigs, weighing between 190 and 200 gm. Each recipient received but a single hypophyseal implant. The recipients were killed 6 to 8 days later. Organs for study were fixed in Zenker's fluid, paraffin sections were made, and stained with hematoxylin and eosin.

This report is based on a total of 30 implants.

RESULTS. GROUP I

Seven animals had small compact ovaries with very few follicles. Their estrous cycles had been characterized by some irregularity in length, by slightly prolonged estrous openings, and indefinite vaginal smears during the estrous openings. The theelin excretion of 4 of these (3, Group I), during the estrous

[1] The irradiation of the animals and most of the implantation experiments were carried out at the Children's Hospital Research Foundation, Cincinnati, Ohio. Some of the preparation of the sections and writing of the manuscript were done at the Marine Biological Laboratory, Woods Hole, Massachusetts.

openings, was found to be less than that excreted by normal animals during estrus, except in 1 animal whose ovaries were functioning almost normally.

The weights of the hypophyses corresponded closely to those from normal animals (normals, average 13.5 mg.; irradiated, average 14.8 mg.) The average weight was less than that of the larger normal hypophyses. The effects of the implants of such hypophyses agreed well with those of normal implants from corresponding periods of estrous cycle, although the stage of the cycle of the irradiated animals was more or less of an estimation, since the time of actual estrus was not always clearly defined by the vaginal smears.

GROUP II

Twenty-three animals had ovaries completely filled with 'interstitial gland,' without any follicles except a few primordial ones and an occasional developing one, and without corpora lutea. Estrous cycles occurred, but irregularly, with prolonged estrous periods characterized by excess cornification or by excessive desquamation of all types of cells associated with vaginal estrus.

The theelin excretion during these estrous periods was usually less than during a normal estrous period, but sometimes equalled it. During a single prolonged estrous period, theelin was excreted at a fairly constant level throughout the period, or there was a maximum point of excretion with little or none the rest of the time (3, Group II). It was concluded, in general, that the prolonged estrous periods were due to secretion of amounts of theelin comparable to or somewhat less than those secreted during normal proestrus.

The weights of the single hypophyses of the irradiated animals were not significantly different from those of normal animals (average of normals, 13.5 mg.; average of irradiated, 14.7 mg.).

Implants were taken from animals at all possible times during the prolonged estrous openings, from the 2nd day open to the 59th day open, and from 4 animals that were open continuously. Only 5 donors were killed during their diestrous intervals. Single normal-estrous implants produced no effects of any kind in the recipients. Single implants from animals with interstitial gland ovaries, taken during their prolonged estrous openings, produced definite follicular stimulation in the ovaries of most of the recipients, and definite vaginal estrus as well as considerable uterine stimulation in all of the recipients.

Twelve Recipients Showing Uniform Responses to Implants from Animals in Prolonged Estrus (Table I).

Ovaries of recipients. All ovaries, except those of 2 animals, showed considerable follicular stimulation, of the same type seen in the normal implant series—rapid growth of small follicles to medium or large-medium ones, or rapid growth of existing medium follicles to large ones, accompanied in some instances by proliferation of the germinal epithelium. These responses were comparable to those after normal-proestrous implants.

Vagina. The vaginal membranes of all ruptured on the 3rd, 4th or 5th day following the implants. All except 2 were considerably enlarged grossly; of these, 11 showed typical estrous changes; only 1 failed to have a full estrus,

but was in a typical proestrous condition when killed on the 6th day. Again, these responses were comparable to those after normal-proestrous implants.

Uterus. All were stimulated, most of them not markedly so, but comparable to the partial estrous reactions seen in some of the normal active series; 2 were more active, more like a complete uterine estrus. Mitotic figures were

TABLE 1. RESPONSE TO IMPLANTS FROM DONORS IN PROLONGED ESTRUS, WITH INTERSTITIAL GLAND OVARIES.

Donors, at time of implant			Recipients, 6 to 7 days after implants				
No.	Vagina; estrous opening and histology	Urinary theelin	Ovaries	Rupture vaginal membrane	Stage of vaginal estrus	Uterus	Mitoses in muscle (av. per section)
153	Sl. open contin. Thin ep. but cornified cells sloughing.	Neg.	Vascular; slight or no foll. stimulation.	3rd day	II–III	Active ep., t.p. and ep. of glands.	71
272	6th day open. Desquamation of mucous, corn., nuc. cells.	Neg.	Many medium and large-med. follicles; 1 large.	4th day	I–II	Partial estrous activity.	0
273	30th day open. Shedding of mucous, nuc. cells, polys.	5[1]	Several large follicles.	3rd day	I–II	Partial estrous activity.	24
277	9th day open. Shedding of mucous, nuc. cells.	4	Several large follicles.	4th day	I–II	Entire t.p. active.	0
283	2nd day open. Surface ep. cells mucified; little sloughing.	Neg.	Many med. and med.-large follicles; germ. ep. prolif.	5th day	I–II	Moderate activity.	9
288	11th day open. Corn., nuc. cells sloughing; polys.	5+	Few med.-large follicles.	4th day	III small	Partial estrous activity.	16
304	19th day open. Corn. cells sloughing.	Neg.	4 large follicles; mitosis in primordial folls.	4th day	I–II small	Partial estrous activity.	0
310	30th day. Corn., nuc., mucous cells sloughing.	5	Number med.-large and 1 large.	4th day	I–II	Partial estrous activity.	47
313	45th day open. Corn., nuc., mucous cells sloughing.	Neg.	Many med. and med.-large follicles.	5th day	I–II very large	Intense estrous activity in entire t.p. Very large.	37
314	32nd day open. Mucous, nuc. cells, few corn., sloughing.	Neg.	Few med.-large; 1 large.	Closed	Early proestrus	Partial estrous activity.	12
315	14th day open. Nuc. cells sloughing.	5 on 8th 9th, 10th days open.	Medium and med.-large follicles.	4th day	I–II	Active estrous changes in entire t.p.	120
318	2nd day open. Mucous cells sloughing.	Neg.	Many med. and med.-large follicles; germ. ep. prolif.	5th day	I–II	Very large; intense activity in entire t.p. and glands.	20

[1] 5 cc. olive oil containing theelin required to bring castrated rat into vaginal estrus (3 or 4 cc. of normal estrous-urine extract are required for rat estrus).

present in the muscle layers in considerable numbers in almost every instance, in contrast to the absence of mitotic figures following normal-estrous implants.

Mammary glands. Almost all showed dilation of the blood vessels, grossly, and slight stimulation of ducts and acini.

Comparison with normal series. Although it is difficult to compare this group with normal-estrous group, it is obvious that a single hypophysis from an animal with interstitial gland ovaries, during the estrous opening, is much more potent than a single or even two-normal-estrous hypophyses. As a whole, the response is comparable to that following a single normal-proestrous or

late diestrous implant, from donors 10 to 15 days after stage I of estrus. It was in this normal group that the maximum response was obtained in ovaries, vaginae, and uteri of the recipients; also, the mitotic proliferation in the uterine musculature reached its maximum here (average number per section, 28). Mitotic proliferation in the uterine musculature following implants from donors in constant estrus was quite variable, from none to many more than in the normal-proestrous series (see table 1); the more numerous ones seemed to be associated with the longer estrous periods of the donors.

Some few might be considered as comparable to the response after normal implants from donors 5 to 6 days after stage I of estrus, where the ovarian stimulation was not very marked and the uterine stimulation slight, including little or no proliferation of the musculature. These donors, though in the midst of prolonged estrus of the more indefinite type, had theelin-free urinary extracts immediately preceding or a few days before the implantation of their hypophyses, indicating a drop in the theelin level comparable to that following a normal estrus.

It can be concluded therefore, that the hypophyses of animals with interstitial gland ovaries are more active during the prolonged estrous openings than are hypophyses of normal animals during estrus. The constancy of the effects of such hypophyseal implants indicate that the prolonged estrous openings are controlled by the same factor, even though individual variation or cyclic variation in this factor causes irregularities in the lengths of the estrous openings and the periods between openings. Since it seems that most of the effects of these implants are comparable to those of early normal-proestrus implants, the conclusions drawn from the study of the theelin excretion are strengthened further —that the prolonged estrous openings are the result of the secretion of subminimal amounts of theelin, comparable to the normal-proestrous or late diestrous amounts.

Six Recipients Showing Variable but Intensified Responses to Implants from Donors in Prolonged Estrus (Table II).

These 6 implants were so markedly effective that the results may be considered separately. There is no explanation, however, for this intensified reaction, for the donors showed the same type of post-irradiation history, and the same histological changes in the ovaries and secondary sex organs, as did the others in Group II. All had ovaries filled with interstitial gland; one had small cysts, one a large cyst, in addition to the glandular tissue, but such variations were also present in the ovaries of the entire group of animals. The amount of theelin excreted, in the entire Group II, showed no correlation with the presence or absence of cysts. All of these 6 had had estrous openings of long duration, but others in the group as a whole had had similarly long periods. Nor was the length of time after irradiation a factor—2 had been irradiated 2 years previously, 4 within the past 9 months; the others in Group II, whose implants caused uniform responses, varied considerably in time since irradiation.

The theelin excretion of all 6 donors was very low throughout the estrous

openings. It was not detectable or just barely detectable in 5 animals; in the 6th, there was a single point of maximum excretion, comparable to the normal estrous quantity, and none during the remainder of the open periods.

Three of these 6 hypophyses caused intense follicular stimulation in the recipients, to a degree not encountered in any of the normal-female series. In one recipient (donor 303), the ovaries were large, and packed with medium, large-medium and larger follicles, such as were found in the normal series and in those of Group II already described; but the total number of follicles here was much greater. In 2 other recipients (donors 286 and 205),

TABLE 2. INTENSIFIED RESPONSE TO IMPLANTS FROM DONORS IN PROLONGED ESTRUS, WITH INTERSTITIAL GLAND OVARIES.

Donors, at time of implant			Recipients, 6 days after implants				
No.	Vagina; estrous opening and histology	Ovaries	Ovaries	Rupture of vaginal membrane	Stage of vaginal estrus	Uterus	Mitoses in muscle (av. per section)
303	31st day open. Nuc. and mucous cells, sloughing.	Glandular; 3 small cysts.	Large; packed with med. and med.-large foll.	3rd day	I–II	Large; early estrous activity.	58
286	59th day open. Pure corn. cells, sloughing.	Entirely glandular; several small cysts.	Large; number of large foll., 2 fully mature.	2nd day	II	Large; postestrous activity.	88
205	33rd day open.[1] Corn. and nuc. cells sloughing, also polys.	Entirely glandular.	Medium size; med. foll., 2 large, 1 mature.	4th day	Thick ep. layer II–III	Large; postestrous activity.	52
307	52nd day open. Mucous, corn. and nuc. cells sloughing.	Glandular; many small cysts.	Large; several large-med. follicles, 3 large; 2 c.l. (ovul. in 1). Germ. ep. prolif.	2nd day	Completed. (repair)	Large; postestrous activity.	180
309	17th day open. Corn. cells sloughing.	Glandular in periphery.	Small; small foll. but 3 c.l. (ovul. in all).	3rd day	Completed. (repair)	Large; postestrous activity declining.	56
203	13th day wide open.[1] Pure corn. and nuc. cells sloughing.	Glandular; 1 huge cyst.	Large; small foll.; 3 c.l. (ovul. in 6).	3½	Completed. (repair)	Large; intense postestrous.	310

[1] Open more or less continuously.

the ovaries contained several similar large follicles, and in addition, 1 or 2 fully mature follicles, such as are found in normal, sexually-mature animals; all were beginning to degenerate, one showed a slight tendency to luteinization. The vaginae of these 3 recipients were enlarged grossly, and vaginal estrus was intense. Their uteri were enlarged greatly, the reactions were those of full estrus; many mitoses were present in the uterine musculature. The reactions as a whole were similar to those obtained following implantation of a single male hypophysis (see normal series), which were greater even than quadruple implants of any normal-female series. Nelson (4) estimated that male guinea-pig hypophyses were 50 per cent more potent than female guinea-pig hypophyses.

Two other hypophyses, from donors 307 and 309, caused a still greater response than the preceding ones. The ovaries of the recipients contained 2 and 3 corpora lutea respectively; ovulation had occurred in all except 1 of the first 2. Vaginal estrus had been completed 6 days after implantation. The uteri were very large, and had had a complete estrous reaction. These reactions

were comparable to or somewhat greater than those following a double male hypophyseal implant.

The 6th hypophysis came from a donor (203) that had been in constant estrus more or less continuously for the preceding 6 months. Although its cyclic history had been no different than that of a number of other irradiated animals, one ovary contained, in addition to the interstitial gland, a huge cyst, measuring approximately 2.1 by 1.9 cm.; the other ovary, containing interstitial gland only, measured approximately 0.4 by 0.3 cm. The ovaries of the recipient contained eight corpora lutea, only 2 of which contained degenerating ova. These corpora looked normal, and were of the size found in normal, sexually-mature animals. (Two to 4 corpora are found usually after a normal estrus.) The vaginal membrane had ruptured in 3½ days, estrus had been completed on the 6th day. The uterus was very large, with characteristic postestrous activity. An enormous number of mitoses were present in the uterine musculature (310 mitoses per section).

The stimulation from all 6 implants was of the follicular type, indicating a greater concentration of the follicle-stimulating substance in the hypophysis, correlated with a continuous but low theelin excretion in the urine. The presence of the large cyst in the ovary of donor 203 was probably responsible for the greatly increased hypophyseal potency. No other animals in Group II had cysts as large as this; small ones seemed to have no influence on hypophyseal activity.

Effects of Hypophyseal Implants from Donors in Diestrus (5 Animals).

The lengths of the diestrous intervals were variable in the same animal and in different animals. Because of this, the time of diestrus could not be compared accurately with the normals. The stage of diestrus was therefore estimated by the structure of the vagina at the time of death.

The implants of 2 donors whose vaginae resembled a proestrous condition, reacted as did hypophyses of normal-proestrous animals, though the uterus of 1 recipient was more active than after any normal-proestrous implant. The implant of a third donor, whose vagina also seemed to be in an early proestrous condition but who had not had an estrus for 103 days, was without effect on the recipient.

The vaginae of the 2 remaining donors were in a resting condition, at 6 and 5 days after a prolonged estrous opening. The former had had leucocytes in the vaginal smear for several days preceding closure. The responses following their implants corresponded probably, to those after a normal implant from 10 days after estrus. One produced some follicular stimulation, typical vaginal estrus and some uterine stimulation. The second produced some follicular stimulation but only vaginal proestrus and slight uterine changes.

It can be concluded therefore, that during the diestrous intervals, hypophyses of animals with interstitial gland ovaries react with about the same intensity as do normal hypophyses at a corresponding period.

SUMMARY

Following irradiation with a minimum sterilization dose of x-rays, the ovaries of some animals functioned normally, but follicles were few, estrous cycles were slightly disturbed in length and vaginal smears were indefinite. Implants of these hypophyses produced responses similar to those following normal implants from corresponding stages of the estrous cycle.

The normal structure of the majority of ovaries, following irradiation, was replaced by 'interstitial gland,' without follicles except for an occasional developing or growing one, and without corpora lutea. Estrous cycles occurred irregularly, with prolonged estrous periods characterized by excessive desquamation of cornified cells or all types of cells associated with vaginal estrus. Theelin excretion in the urine was usually less than during a normal estrous period.

A single normal-estrous hypophyseal implant produced no response in recipients. Most (12) single implants, from donors with interstitial gland ovaries, in prolonged estrus, produced responses similar to those following single normal proestrous or late diestrous implants. A few implants (6) were much more potent, producing intense follicular growth and formation of corpora lutea, comparable to the response following single or double male hypophyseal implants (normal), and in one instance, a response many times greater than this.

Diestrous implants reacted as did normal implants from corresponding stages of diestrus.

I am indebted to Dr. L. H. Schmidt for assistance with the implantation experiments.

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EFFECT OF EMMENIN ON GONADOTROPIC HORMONE EXCRETION IN CASTRATES AND SPONTANEOUS MENOPAUSE

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A number of preceding investigations (1, 2, 3) give evidence that the hypophysis during the menopause is over-acting and flooding the system with excessive quantities of follicle-stimulating and luteinizing hormones. We have been guided in arriving at this conclusion by assays of the blood and urine of such patients, continued over long periods of time.

Additional objective criteria have been the vaginal spreads of the patient taken throughout the time of investigation (4). These vaginal smears are commonly negative (leucocytes with occasional deep cells) but in some patients permanently resemble the positive phase—squamous cells with large nuclei, deep ovoid cells with large nuclei and occasionally leucocytes.

In addition, such patients complain subjectively of neurovascular symptoms, flushes, sweats and headaches, arthritic pains, as well as digestive symptoms. As these symptoms are purely subjective, less importance has been conceded to them in our investigations.

Our studies with dihydrotheelin benzoate (estradiol benzoate)[2] given by injection (3) as well as dihydrotheelin tablets, 600 active 'biological units' given by mouth (3),[2] have shown convincingly that the oversecretion of anterior pituitary hormones is controlled by this medication if given in sufficiently large dosage. While this therapy is employed, both follicle-stimulating and luteinizing hormones disappear from the circulation and urine. The vaginal spreads become definitely positive, changing either from the negative phase or from the pseudo-positive phase. The really positive spread is characterized by the decrease in size of the nuclei of the squamous cells. There is as well the complete disappearance or amelioration of the subjective symptoms complained of. On discontinuing the treatment, the symptoms either rapidly or gradually reappear, coincident with the reappearance of the prepituitary hormone in the blood and urine, and the return of the vaginal smear to its previous state.

In the present study, emmenin (Collip) was employed instead of progynon. Emmenin, which is the lipoid extract obtained from the human placenta, has now been purified to a crystalline form by Collip (5). The evidence supplied by Cohen and Marrian (6) as well as by Cohen, Marrian and Odell

[1] Hiram N. Vineberg Research Fellow in Gynecology.
[2] Both progynon B and D H were available to us through the kindness of Drs. Gregory Stragnell and Edwin Schwenk of the Schering Corporation, Bloomfield, New Jersey.

warrants the conclusion that emmenin is an estriol glucuronide. Collip has always emphasized that while emmenin exerts a strong estrogenic action in the immature rodent with ovaries present, only a fraction of this action is noted in the castrated adult rodent. The assays reported by Cohen, Marrian and Odell (7) with pure substance confirm this. These authors found that emmenin contains 350 M.U. per mg. when titered on castrated adult rats while the same weight of uncombined estriol develops 10,700 M.U. This signifies that emmenin in the castrate exerts only 1/17 of the effect of estriol. It explains in great part the difference in assay of some urines (especially urine of pregnancy) if the so-called ether insoluble 'combined' estrogenic factors are not hydrolized before lipoid extraction (6).

The present study was undertaken to determine *a)* whether emmenin given in the menopause inhibited the over-secretion of the hypophysis; *b)* whether, in the human subject, the absence or presence of the ovary, influenced such reaction if obtainable.

Three cases were studied, two surgical castrates and one spontaneous menopause. Gonadotropic hormone determinations were performed on 48 or 72-hour specimens according to the acetone precipitation method previously described (8). In each case the titres were studied over a preliminary period of 14 to 19 days without treatment. The patients were then given a concentrated oral preparation of emmenin in capsule form. Each capsule contained the equivalent of approximately 110 oral units of the liquid emmenin.[3] By using this concentrated preparation in capsules the patients were able to tolerate doses equivalent to 11½ drams and 16 drams of the liquid emmenin per day for a period of weeks, without gastric upset.

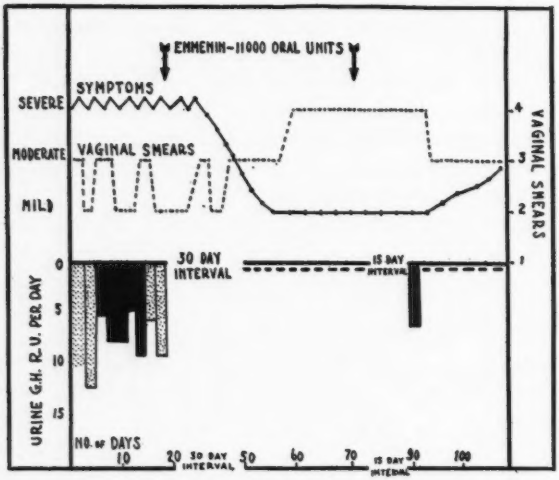
Case 1, 45 years of age, was castrated surgically 6 weeks before the beginning of the present study. Preliminary daily gonadotropic hormone determinations were performed for 19 days. Both follicle-stimulating and luteinizing factors were found in the preliminary specimens, the daily excretion varying from 5 to 12 R.U. a day. The patient was then given 11,000 U. of emmenin over a period of 54 days. For the first 30 days of the period of treatment no gonadotropic hormone determinations were performed. Following this 30-day interval, gonadotropic hormone studies were continued for 24 days. During this 24-day period the urine was found to be consistently negative for gonadotropic hormone. An interval of 15 days was then allowed to elapse and thereafter urine gonadotropic hormone studies were again performed for an additional 18 days. During the latter period, one specimen (16th day after stopping the emmenin) contained 5 R.U. of luteinizing factor per litre. All other specimens were found negative for gonadotropic hormone.

The patient reported definite symptomatic improvement coincidentally with the disappearance of the gonadotropic hormone from the urine. Six weeks later there was still no recurrence of symptoms. The vaginal spreads of this patient before treatment were found to be pseudo-positive. During treatment they become positive in 40 days; after the therapy was discontinued the spreads

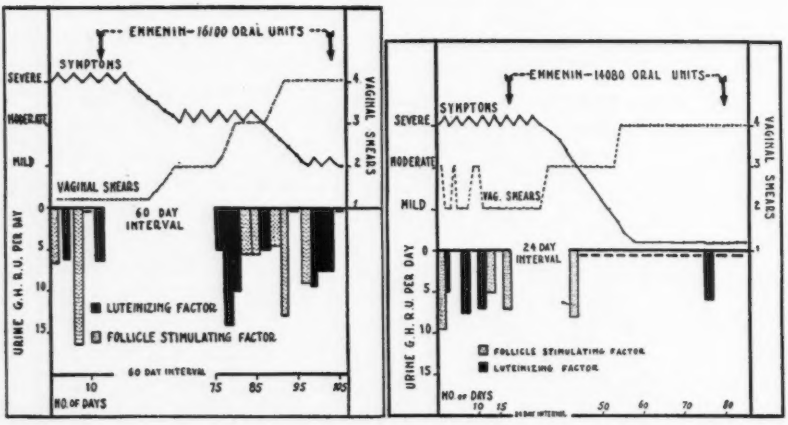
[3] We are indebted to the Ayerst, McKenna and Harrison Company of Montreal, Canada, for the generous supply of concentrated emmenin used in this investigation.

remained positive for 14 days. This castrate received treatment 2 months after castration and showed rapid and satisfactory response.

Case 2, 58 years of age, was surgically castrated 10 years before the



Case 1



Case 2 Case 3

Effect of emmenin on gonadotropic-hormone excretion, vaginal smears and symptoms. Case 1, *surgical castrate,* 45 years old, operation 6 weeks before. Case 2, *surgical castrate,* 58 years old, operation 10 years before. Case 3, *natural menopause,* age 47 yr., menopause since 6 months before.

present study. The preliminary period of gonadotropic hormone determinations extended over 14 days. The excretion of gonadotropic hormone during this control period varied from 6 to 16 R.U. per day. The patient was then given 16,100 U. of emmenin over a period of 90 days. During the first 60 days no gonadotropic hormone studies were performed. Thereafter, the daily

gonadotropic hormone excretion was determined for 30 days. In this patient no appreciable diminution in the amount of gonadotropic hormone factors excreted was noted. The vaginal spreads which were negative before treatment became positive during the treatment. Emmenin in this case, although given in appreciably larger dosage than in case 1, failed to influence the gonadotropic hormone excretion. The patient reported moderate improvement in her symptoms while she was taking the emmenin. The symptomatic improvement, however, was neither as striking nor as lasting as in Case 1 (M93) in which the emmenin produced disappearance of gonadotropic hor-

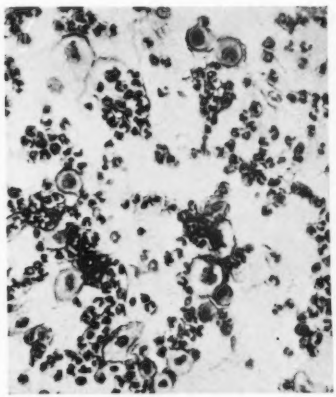


Fig. 1

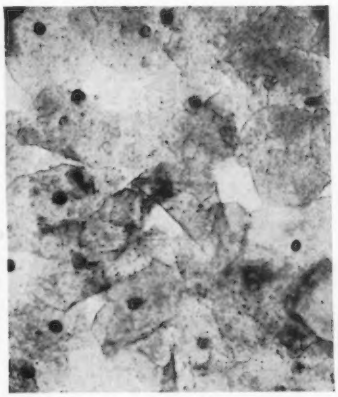


Fig. 2

Fig. 1. Vaginal spread from case 2, surgical castrate, before treatment. Negative, consisting of leucocytes and occasional ovoid nucleated deep cells.

Fig. 2. Vaginal spread from case 2, surgical castrate, after treatment was instituted. Positive, showing change to non-nucleate or small, nucleate, squamous epithelial cells.

mone from the urine. The spreads, however, remained positive for 5 weeks after discontinuing the treatment.

Case 3, age 47 years, was a case of natural menopause of 6 months' duration. During the preliminary period of study of 18 days the excretion of gonadotropic hormone factors varied from 0 to 10 R.U./day. The patient was then given 14,080 U. of emmenin over a period of 60 days. No gonadotropic hormone studies were performed during the first 24 days of treatment. Thereafter the daily gonadotropic hormone excretion was followed for 44 days. With the exception of 2 specimens (25th and 57th days after the emmenin was started), the urine was found uniformly negative for gonadotropic hormone. The vaginal spreads became positive 27 days after the beginning of treatment. This patient reported striking relief of her symptoms 14 days after the beginning of treatment. The symptomatic relief continued throughout the period of observation, the spreads remaining positive for 2 months after stopping the medication.

From the present study it would appear that emmenin in adequate dosage (case 1) like other estrogenic substances, can produce disappearance of excessive hypophyseal excretion. In case 2, a castrate 10 years after operation, al-

though a larger amount was employed, the adequate dosage necessary for this patient was evidently not attained. Such difference in response was noted by us in a larger series treated with dihydrotheelin benzoate, a much more powerful and more quickly acting estrogenic substance (3). The full effect in case 1 was obtained in a patient with ablated ovaries. The evidence is insufficient to decide conclusively whether such variation in result is accidental or due to absence of the ovaries, which Collip, in his animal experiments, found a decisive factor in obtaining estrogenic results with emmenin. In case 3 excretion of gonadotropic substance was inhibited from the 45th to the 75th day when it reappeared for 2 days. This signifies that almost an adequate dose had been given.

From these observations it seems warranted to conclude that emmenin, like other estrogenic substances, in very large dosage, can produce disappearance of the over-excretion of gonadotropic substance observed in the urine of menopause patients, both castrate and spontaneous. Its action is slower than that of some of the other estrogenic substances. Whether the action is more prolonged, was not determined in this investigation.

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THE EFFECT OF HYPOPHYSEAL INJECTION AND IMPLANTS ON THE ACTIVITY OF HYPOPHYSECTOMIZED RATS

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The present experiments were undertaken to determine whether rats made inactive by hypophysectomy could be restored to normal behavior by daily injections of fresh hypophyseal tissue, or by hypophyseal implants. Observations were made simultaneously on the effects produced by these forms of replacement therapy on body weight, food, and water intake and the reproductive tract, as well as on the glands of internal secretion.

METHODS

The rats were kept in separate activity cages containing a revolving drum with a cyclometer and a living compartment with a food-cup and an inverted graduated water bottle. Readings were made daily of activity, food intake (standard McCollum diet), water intake and vaginal smears, and weekly of body weight. Most of the animals were approximately 50 days of age when they were placed in the cages. They were hypophysectomized 10 to 20 days later when a fairly high and constant level of running activity had been attained; 40 days after the operation, when they were approximately 100 days of age, they were killed and autopsied. Replacement therapy was started on the day of hypophysectomy.

The hypophyses used for the daily injections were taken from animals averaging 150 days of age. They were cut into very fine pieces, emulsified in physiological salt solution and injected subcutaneously with a bevel-edged glass cannula. The injections were made in the back where the injury would give least interference with running activity. The same hole in the skin was used for several successive injections.

The implantations were made in the anterior chamber of the eye according to the technique used by Buxton (1) for hypophyseal implants and by us for parathyroid implants (2). A whole gland from a donor approximately 12 days of age was implanted into one eye. At autopsy the implants were removed, fixed in Bouin's solution, sectioned and stained with eosin and Poirier's Blue after the method of MacCallum. Also, all of the glands of internal secretion, the adrenals, gonads, thyroid, thymus, pancreas and uterus were removed, weighed, and preserved for histological study. The spleen was similarly treated. Finally, the completeness of the hypophysectomy was determined by a search for glandular tissue in the hypophyseal pouch of the injected brain under a dissecting microscope.

TABLE 1.

Rat No.	Age at operation. Days	Activity Av. rev./day		Body wt. Gm.		Food intake Av. gm./day		Water intake Av. cc./day	
		10 days before operation	10–20 days after	At operation	20 days after	10 days before operation	10–20 days after	10 days before operation	10–20 days after
A. Hypophysectomized female controls.									
168 J	74	9,015	784	183	146	15.0	6.1	24.2	24.7
167 J	74	7,159	548	188	134	15.9	4.6	23.6	14.6
183 J	65	14,221	1,412	138	118	14.4	5.6	20.7	20.6
177 J	59	6,266	520	118	99	10.4	6.1	17.2	17.9
176 J	59	6,046	912	135	113	11.9	7.0	17.5	20.7
195 J	62	4,437	1,627	137	128	13.3	7.1	22.8	21.8
181 J	65	11,190	1,227	160	130	14.1	7.5	26.0	21.0
Mean	65	8,333	1,004	151	124	13.6	6.3	21.7	20.2
B. Hypophysectomized males with daily ant. lobe injections.									
24 D	62	3,634	849	136	149	13.4	9.2	24.6	17.0
14 D	75	1,966	825	242	228	16.3	11.8	25.5	18.9
250 G	60	7,179	11,384	160	182	16.2	12.6	25.3	33.2
105 G	79	4,039	1,163	220	197	15.2	11.0	27.1	16.8
104 G	79	6,434	1,615	228	215	16.3	12.6	26.1	21.4
103 G	87	947	25	260	230	17.8	12.1	25.0	18.0
Mean	74	4,033	2,644	208	200	15.9	11.7	25.6	23.3
C. Hypophysectomized females with daily ant. lobe injections.									
225 I[1]	106	22,409	4,112	185	187	15.7	9.9	35.9	28.3
113 G[1]	87	16,930	575	165	181	14.5	10.9	24.8	20.4
115 G[1]	81	12,910	5,270	179	188	17.4	12.9	31.4	23.6
259 I[1]	86	19,111	12,120	185	162	17.8	12.2	30.7	26.8
166 J[2]	58	15,622	5,038	143	166	14.8	12.3	16.3	19.7
107 J[2]	99	9,814	578	175	197	13.6	14.4	31.5	67.8
127 J[2]	94	13,253	605	198	208	15.3	12.1	66.6	60.3
Mean	87	15,721	4,043	176	184	15.6	12.1	33.9	35.3
D. Hypophysectomized females with implants to eye.									
77 J	74	17,040	1,574	166	143	13.2	8.0	31.1	40.6
81 J	67	17,561	6,093	141	129	13.2	6.4	21.4	21.4
91 J	74	10,926	1,100	165	144	11.3	6.2	27.0	19.1
102 J	47	8,947	5,051	110	106	9.9	7.7	20.8	16.6
104 J	47	6,991	2,973	116	116	9.6	6.3	18.5	16.1
379 I	63	6,736	1,199	154	132	10.2	7.0	26.0	15.2
17 J	71	6,241	740	154	131	10.8	6.7	21.8	15.5
22 J	58	9,306	5,540	150	134	13.2	7.3	21.0	20.7
12 J	74	8,799	5,470	171	158	13.8	8.6	28.6	26.5
11 J	74	6,626	965	176	147	12.4	6.7	16.0	16.3
366 I	76	3,308	632	180	160	13.5	7.3	34.4	25.1
353 I	65	2,552	695	159	139	13.9	8.8	18.9	19.2
387 G	65	3,413	1,721	156	140	14.2	9.0	18.2	19.2
304 F	76	2,684	453	161	150	9.7	6.4	17.8	27.6
Mean	67	7,938	2,443	154	138	12.1	7.3	23.0	21.4

[1] One anterior lobe injected daily.
[2] Two anterior lobes injected daily.

RESULTS

A. Control Experiments on the Effects of Hypophysectomy. In confirmation of earlier experiments (3) hypophysectomy was found to produce an abrupt decrease in running activity to a very low level, a definite decrease in food intake, and a decrease in body weight. It produced a transient increase in water intake in some animals and ultimately a small but permanent decrease in all of them. A typical record from 1 of the 7 control animals is presented in fig. 1, *A*. Activity dropped from an average level of 11,190 revolutions per day for the 10-day period before hypophysectomy to a level of 1,227 revolutions 10 to 20 days later. Food intake decreased from 14.1 gm. to 7.5 gm. in the same time, and body weight from 160 to 130 gm. Water intake, not shown on the chart, showed only a small decrease.

The results of these control experiments on all 7 female rats hypophysectomized at an average age of 65 days are summarized in table 1. Averages are given for the 10-day period preceding hypophysectomy and for the 10-day period included between the 10th and 20th day afterwards. This latter period was chosen for the reason that by the 10th day after hypophysectomy activity, food and water intake ceased to show any more changes. The levels present during this period may be taken as representative of the permanent effects. It was important to make the anterior-lobe injection experiments as short as possible in order to reduce the number of animals that had to be sacrificed for their hypophyses.

In the period of 10 to 20 days after hypophysectomy the activity decreased 88 per cent, body weight decreased 18 per cent, food consumption decreased 54 per cent and water intake decreased 7 per cent from the corresponding levels before hypophysectomy.

B. Effects Produced by Daily Injection of Fresh Anterior Lobe Substance. Daily injections of fresh anterior lobe tissue were started in 13 animals on the day of hypophysectomy and continued for a period varying from 17 to 80 days. Activity was increased in only 1 instance, while in 4 it showed a definite replacement effect with a decrease much less marked than in the untreated animals. The record of the one animal which showed full replacement of activity is presented in fig. 1, *B*. Treatment with 1 gland per day was started on the day of hypophysectomy. Activity decreased markedly but temporarily from the preoperative level of 7,179 revolutions per day, and then increased rapidly to a level of 11,384 revolutions at which it was maintained for 15 days until the injections were stopped. Discontinuance of treatment was followed by a sharp decrease to a level of comparative inactivity characteristic of untreated hypophysectomized animals. Body weight increased during the period of injection and decreased afterwards. Food intake was maintained at a slightly reduced level during the period of injection, and showed a sharp drop to a considerably lower level after they were stopped. Water intake, not shown in the charts, remained fairly constant throughout. The results of these experiments are summarized in table 1, *B* and *C*, which gives the records of 6 males and 7 females. All of the males received injections of 1 gland per day. It will be seen that one animal was made more active by the injections.

The average daily activity of the 7 females was decreased 74 per cent in the 10 to 20 day period after hypophysectomy. It will be noted that despite the small difference in percentage decrease compared with that of the untreated animals, in 4 of the 7 the 10 to 20 day level was considerably higher than that of the untreated group. The number of glands, whether 1 or 2, received daily by each animal is shown in the table.

Full activity replacement by anterior lobe treatment was thus obtained in 1 animal and partial replacement in 4. The failure to obtain full or even partial activity replacement in a larger number of animals cannot be explained at present. The increase in dosage to 3 glands per day in several animals did not

TABLE 2. COMPARATIVE GLANDULAR WEIGHTS, IN MILLIGRAMS

Group	No. of rats	Age at operation. Days	Age at autopsy. Days	Thyroid	Ovaries	Uterus	Adrenals	Pancreas	Thymus	Spleen
Normal females	6	—	100	19.0	68	377	33	977	311	576
Hypophysectomy with injection of 2 ant. lobes per day	3	84	107	20.5	225	402	32	896	148	836
Hypophysectomy with injection of 1 ant. lobe per day	4	90	137	21.0	82	342	27	—	273	941
Hypophysectomy with whole hypophysis implant to eye	16	67	103	14.0	33	101	19	485	237	373
Hypophysectomized untreated controls	7	65	93	12.4	32	126	12	485	122	328

produce a greater increase in activity. Injuries and discomfort produced by the daily injections may be partly responsible, but, as will be shown below, the failure of the injections to maintain normal growth of some of the endocrine glands, particularly the adrenals, may also be responsible.

In marked contrast to activity, body weight showed an increase over the preoperative level in 6 of the 7 females, with an average increase of 5 per cent for the group. Four of the 6 males showed a decrease with an average decrease of 4 per cent for the group. Food intake showed an increase in 1 animal and a decrease in 12. This represents an average decrease of 24 per cent, which is much less than that of the untreated animals (54 per cent). The postoperative level (11.9 gm.) was considerably above the intake of untreated animals (6.3 gm.).

Water intake was unaffected except in 1 animal on the 2-gland dosage. This animal showed a greatly increased intake immediately following hypophysectomy (31.5 to 67.8 cc.), but unlike the others failed to show the usual immediate decrease characteristic of totally hypophysectomized animals. On the contrary its water intake was maintained very steadily at this increased level just as it is in animals in which the posterior lobe is removed leaving

some anterior lobe tissue intact. The anterior lobe injections clearly maintained the polydipsia for the total removal of the anterior lobe was confirmed at autopsy.

The effects produced on the reproductive tract as judged from the vaginal smears were rather irregular. Four of the animals showed constant cornification of the cells; 2 showed irregular periods of prolonged cornification; and 1 showed a complete absence of cornification. In 1 animal, raised from 1 to 2 glands per day, the vaginal smears changed from continuous cornification to epithelial cells and leucocytes. We are unable to explain this phenomenon in view of the great and uniform hypertrophy of the ovaries disclosed at autopsy.

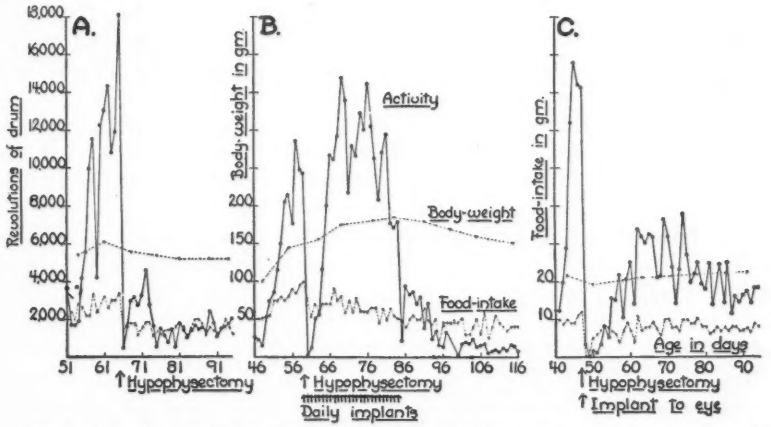

Fig. 1. *A.* Record of animal showing the effects produced by hypophysectomy on activity, body weight and food intake. *B.* Record of hypophysectomized animal showing effects produced by daily injections of fresh anterior lobe emulsion on activity, body weight and food intake. *C.* Record of hypophysectomized animal showing effect of hypophyseal implants to eye on activity, body weight and food intake. Implant was made immediately after hypophysectomy.

A postmortem study was made of the weights of the other endocrines in the hope of determining further the degree of replacement produced by this form of treatment and at the same time possibly throwing some light on the failure to obtain full activity replacement. The summary of the autopsy findings is contained in table 2 which gives the average weights for 6 normal females, 7 hypophysectomized untreated females, and for 2 groups of females treated with anterior lobe injections, 1 group receiving 1 gland per day, the other, 2 glands. Unfortunately, we were unable to autopsy all the animals at the same age; the differences, however, are probably not too great for the present purposes. The thyroid showed a slight hypertrophy with both doses (20.5 and 21.0 mg. as compared to 19 mg. for normals); the ovaries showed a very marked hypertrophy with both doses; the uterus showed an hypertrophy with the 2-gland dose and a slight atrophy with the 1-gland dose; the adrenals were of normal weight with 2 glands and approximately four-fifths normal with 1 gland; the pancreas became slightly atrophic with 2 glands; the thymus gave

very inconstant results; the spleen showed a marked hypertrophy with both 1 and 2-gland doses.

It is clear from these data that daily anterior lobe injections have the greatest effect on the ovaries and the next greatest on the thyroid; the adrenals and the pancreas are least affected. It is possible that the failure to obtain full activity maintenance may have been due to the inadequate function of the adrenals and the pancreas. The fact that both activity and food intake are markedly influenced by the adrenals (4), and less so by the pancreas (unpublished data), would indicate that the failure to obtain full activity was due largely to the lack of sufficient adrenotropic hormone.

It is of considerable interest that the food intake of hypophysectomized animals treated daily with injections of anterior-lobe emulsion did not remain consistently on a higher level, but showed definite and regular cyclical fluctuations (see fig. 2). These variations from 3 to 16 gm. food intake per day recurred at intervals of 6 to 11 days. These fluctuations are of particular interest inasmuch as in previous experiments similar cycles, observed in food and water intake after section of the pituitary stalk (5), were completely abolished by removal of the ovaries. It is very likely that the cycles of this animal with the anterior lobe injections would also have disappeared after removal of the ovaries.

C. Effects Produced by Hypophyseal Implants to the Anterior Chamber of the Eye. It was known from the work of Buxton (1) that hypophyseal implants to the anterior chamber of the eye show definite viability for at least several weeks, but the physiological effects produced by these implants had not been studied. In the present experiment 14 animals received implants (donors 12 days old) on the day of hypophysectomy. Of this number 13 showed either functional or histological evidence of viability of the grafts. The basophils, eosinophils and chromophobes were present but did not stain well.

None of the animals showed full maintenance of activity. However, 4 showed a post-operative level definitely above the low level of untreated animals. A record of 1 of the animals showing an activity effect is presented in fig. 1, *C*. Activity after hypophysectomy was maintained at a level of approximately 5,000 revolutions per day, which is considerably above that of untreated animals. Body weight increased slightly, while food intake showed a decrease. The vaginal smears presented a continuous diestrous picture.

The results of these experiments are summarized in table 1, *D*. As before, the 10 to 20-day period following hypophysectomy was used for test purposes. The decrease in activity was 69 per cent, which is less than that of the female group treated with anterior-lobe injections. It will be noted, however, that the average for the test period was only 2,443 as compared to 4,043 revolutions for the anterior-lobe-injection group of females. Only 4 animals gave averages which were considerably above the untreated post-operative level. Body weight showed a decrease in all of the 14 animals, averaging 154 gm. for the period before hypophysectomy and 138 gm. for the test period. Food intake attained a level slightly above that of the untreated group (7.3 grams as compared to 6.3 grams), but all of the animals showed a definite decrease.

Water intake increased in 5 animals, decreased in 8 and remained unchanged in 1 but the averages for the 2 groups (treated and untreated) were practically the same (20.2 and 21.4 cc.).

In contrast to the daily anterior lobe injections the implants had a relatively greater effect on activity than on food intake, body weight, or the reproductive tract. It became of interest to determine whether an explanation of these differences could be obtained from a study of the replacement effects on the different endocrine glands. The results of these observations are summarized in table 2. The ovaries and uterus showed no effect at all (ovaries 33 mg. as compared to 32 mg. for the untreated animals; and uterus 101 mg.

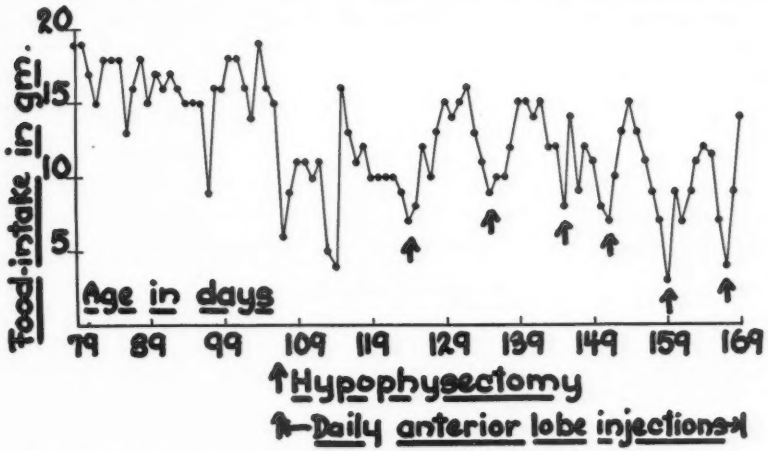

Fig. 2. Record showing cyclical variations in food intake present in a hypophysectomized animal during period of daily injections of fresh anterior lobe emulsion.

as compared to 126 mg.); the pancreas and spleen were unaffected; a slight increase over the untreated level was shown by the thyroid, and a larger increase by the adrenals. These results indicate that hypophyseal implants have a greater adrenotropic and thyrotropic than gonadotropic effect.

It is possible then that the increase in activity above the level of untreated animals was due either to the adrenotropic or the thyrotropic action of the implants. Of the two the adrenotropic effect seems to be the more important. The result of the daily anterior lobe injection experiment also pointed to a close relationship between activity and the adrenotropic effect.

DISCUSSION

It has now been shown that hypophysectomy produces, in addition to the marked atrophy of the thyroid, gonads, adrenals and pancreas, a great and permanent decrease in spontaneous activity. Inasmuch as an equally great decrease in activity can be produced by removal of any one of these glands it would follow that a successful replacement therapy in hypophysectomized animals probably would have to maintain normal function of them all. Experi-

ments are now in progress on the effects produced by extracts of the different glands given singly and in various combinations. It has been found that body weight and food intake can be fully maintained by growth hormone while activity remains entirely unaffected on a flat low level. It is clear that in order to obtain full replacement any therapy must produce normal activity as well as normal growth and sex development.

SUMMARY

Hypophysectomy of 7 control females produced a marked decrease in spontaneous activity, food intake and water intake. Daily injections of emulsion of fresh anterior lobe substance started on the day of hypophysectomy greatly reduced the amount of the decrease in activity, food intake and water intake in 4 of 13 animals, but produced full replacement in only 1 animal. Hypophyseal transplants to the anterior chamber of the eye showed definite viability in 13 of 14 attempts, but had a small stimulating effect on activity, food and water intake in only 4 animals. Daily injections of fresh anterior lobe emulsion had the greatest effect on the ovaries, the least on the adrenals; implants to the eye had no effect at all on the ovaries but a definite effect on the adrenals. Replacement therapy to be complete must maintain normal activity and food intake as well as body weight and the reproductive tract.

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THE EXCRETION OF GONADOTROPIN BY NORMAL HUMAN MALES AFTER THE INGESTION AND INJECTION OF EXTRACTS OF PREGNANCY URINE

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The recent finding of gonadotropic activity in plants (1, 2, 3) prompts the question of the importance of such plants in nutrition and their possible physiological significance relative to the pituitary hormone. If such plant gonadotropins are of physiological significance one might expect that they would, upon ingestion, produce some biological effect or lead to an increased rate of formation of the pituitary gonadotropic hormone.[2] In the latter event an increased urinary elimination of gonadotropin might reasonably be anticipated in view of the readiness with which the mammalian kidney excretes P.U.[3] However, one might just as reasonably expect similar results from the oral ingestion of large amounts of P.U. With such questions in mind the present experiments were conducted.

METHODS

Subjects and samples. Four males with no detectable abnormality served as subjects. The collections were started in the morning following bladder evacuation, and carried through until the same hour on the following morning, during which time the collected urine was kept in the refrigerator. When P.U. was administered, it was given just before starting the 24-hour collection. In some of the later experiments the samples were not handled individually. On these occasions the 24-hour samples of 2 individuals were combined, and then divided into 2 parts for extraction and assay. Consequently, some of the values obtained do not represent the excretion of one person, but the average 24-hour output of two persons.

Extraction procedures. The benzoic acid technique was that of Katzman and Doisy (8), including 1 washing of the initial precipitate with saturated aqueous benzoic acid, and 2 washings with 10 volumes of cold acetone. The centrifuged residue was twice extracted with alkalinized water (barely pink to phenolphthalein) for about 90 minutes each time. After removing the insoluble material by centrifuging, the combined extracts were adjusted to pH 6.5 and precipitated from 10 volumes of cold acetone. The precipitate was then air dried under reduced pressure, and dissolved in water with the addition of sufficient dilute alkali to bring the reaction just beyond neutrality (phenolphthalein). After discarding any insoluble

[1] This work was supported by a grant from the Rockefeller Foundation.
[2] Although Dickens (4) and Ehrhardt (5) reported some evidence of activity of orally ingested P.U. in immature rats, we could not secure similar results in the mature rabbit (6). The administration of 5-10,000 R.U. of P.U. in 1 dose added to a small portion of the food ration did not lead to any detectable ovarian changes. For several reasons, however, these negative results are not decisive, nor are those of Grollman and collaborators (7).
[3] Abbreviation for pregnancy urine gonadotropin.

matter the resulting solution was neutralized, made to 20 cc., and injected into rabbits for assay. This procedure resulted in extracts which could be given intravenously in 1 dose depresenting a total 24-hour sample. In the 6 instances in which this was done, none of the injected animals evidenced any significant distress. It should be emphasized, however, that the omission of either the washing with saturated aqueous benzoic acid, the washing with cold acetone, or the reprecipitation from acetone, is not feasible as the toxic products of our preliminary extractions would bear testimony.

While our first experiments were under way, Dr. Louis Levin kindly informed us of the details of his tannic acid method (9), and generously gave us permission to employ this method in our work. Consequently, in some of the later experiments the tannic acid method was used either alone, or for parallel determinations on composite specimens. Due to the somewhat lower toxicity and higher activity of the tannic acid product it is probably unnecessary to reprecipitate the material from 10 volumes of acetone. However, to make the two methods more nearly comparable from the standpoint of yields, the tannic acid procedure was conducted exactly like the benzoic acid procedure with the following modifications. Ten cc. of 20 per cent aqueous tannic acid solution per liter of urine served for the precipitating agent in place of benzoic acid. The precipitate was washed only with acetone (twice), the step of the aqueous washing being omitted.

Assay. The assays were conducted on postpartum rabbits known to be free from snuffles and ear tick, and whose good health was attested by their daily food consumption, maintenance or gain of body weight, and their excellent reproductive performance. The method has been described before (10), and the reliability of the results checked by the data of Rowe, Simon and Nelson (11). The rabbit unit is defined as the quantity per kilogram of body weight required to cause ovulation in a postpartum rabbit. For P.U. the ovulating dose is 1 rat unit per kilo; hence for this product the rabbit and rat units are identical.[4]

P.U. Extract. The material employed for these experiments consisted of several samples of especially prepared antuitrin-S, furnished through the courtesy and cooperation of Dr. Oliver Kamm, of Parke Davis and Company. The samples used for the ingestion experiments were in the dry form, and were made up in distilled water immediately before use. The P.U. employed in the injection experiments was supplied in sterile solution. At the time of each experiment in which P.U. was used, the potency of the preparation ingested or injected was checked by assay on rabbits. The dry P.U. assayed 225 R.U. per mg. At the time of the first injection experiment, the P.U. solution assayed 165 R.U. per cc. Several months later when the last injections were made, this solution assayed 120 R.U. per cc.

RESULTS

A. Daily Excretion of Gonadotropin by Normal Males. In figure 1 the values obtained by the benzoic acid and tannic acid methods are indicated by the unshaded squares. It may be noted that the 15 yields obtained with the benzoic acid method on 18 urine specimens (3 composite samples) vary about a mean value of 6 U, being greater than 12 U only twice. The 11 determinations on 15 samples by the tannic acid method (4 composite samples) gave more variable results, so that no mean value can be ascertained from the relatively few data. It is to be seen, however, that in general the tannic acid

[4] For example, 3 rat units of P.U. are required to provoke ovulation in a rabbit weighing 3 kg.

values are higher than those obtained with the other method. See figure 1.

B. Excretion of Gonadotropin after the Oral Ingestion of P.U. Benzoic acid determinations were made on two 24-hour specimens[5] after the ingestion of 8,000 R.U. of P.U. orally, on 6 specimens after the ingestion of 10,000 U, and on 2 specimens after 20,000 U. On 3 composite samples of 6 of the above specimens, parallel tannic acid determinations were made. Finally, 2 specimens were examined by the tannic acid method after the ingestion of 42,500 U. In no instance did the values obtained by either method fall outside of the normal range. In some of the assays on the above mentioned specimens, we were content to demonstrate that the 24-hour excretion was below the upper limit of the normal range, and no lower limit was determined. However, on 6 benzoic acid extracts representing 9 specimens, and on 1 tannic acid extract representing 2 specimens, the assays were conducted so as to determine both upper and lower limits. These results are indicated by the shaded squares in figure 1.

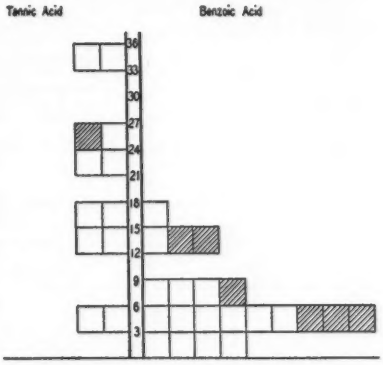

FIG. 1. Daily excretion of gonadotropic substance by normal males as determined by the benzoic acid and tannic acid methods. The numbers in the middle column indicate RB.U. per 24 hours. Each unshaded square represents an assay on an individual or composite specimen. The shaded squares represent a few of the determinations on urine from the same subjects after the oral ingestion of P.U.

C. Comparison of Values Obtained by Parallel Benzoic Acid and Tannic Acid Determinations on the Same Urine Samples. On 6 of the composite specimens, representing either the normal excretion, or the excretion following the ingestion of P.U., as discussed under section *A* and *B.*, parallel extractions were made. The results of these determinations are shown in table 1. In 4 of the 6 specimens the tannic acid values were distinctly higher; in no case was the reverse true.

D. Elimination of Gonadotropin Following the Intramuscular Injection of P.U. The specimens in these experiments were divided into halves and examined by both methods, or, 2 (or more) specimens were mixed and aliquot portions taken for parallel determinations. In any event, the quantity of urine used for each process was never less than the equivalent of 12-hours elimination. The results are listed in table 2. It is apparent that the intramuscular injection of 480 R.U. of P.U. was not followed by any significant increase in the excretion of gonadotropin by the 4 subjects. The experiments with the higher doses were conducted on only 2 of the 4 subjects (*F.* and *W.*). A cursory examination of table 2 gives the impression of an utter lack of consistency in the results. It should be noted, however, that the experiments are listed in chronological order. When the data secured from subjects *F.* and *W.* are examined chronologically, it is seen that the average benzoic acid yields

[5] The urine was collected for 2 successive 24-hour periods in this experiment. The gonadotropic excretion in the second 24-hour period likewise fell within normal limits.

following the injection of 600 U, or more, show a progressive increase, while the reverse is true of the tannic acid yields. Disregarding these progressive changes for the moment, the data indicate that the injection of as much as 500 R.U. of P.U. into the body need not be followed by any appreciable urinary elimination of gonadotropin above the normal limits. The injection of 600 to 750 U intramuscularly may be followed by the excretion of 5 to 15 per cent of the activity.

DISCUSSION

The negative results of the experiments with orally ingested P.U. are in agreement with the similar results of Ehrhardt (5) obtained on unconcentrated urine. Still, the failure of orally ingested P.U. to increase the urinary excretion of gonadotropin is of very little significance in view of the results obtained after the injection of 480 R.U. of P.U. intramuscularly. Even if the rate of absorption from the gastrointestinal tract equalled that from the injection site,

TABLE 1. ASSAYS OF PARALLEL BENZOIC ACID AND TANNIC ACID EXTRACTIONS OF THE SAME COMPOSITE URINE SAMPLES, EXPRESSED IN RB.U. PER 24 HOURS.

Sample	Benzoic Acid		Tannic Acid	
1		< 8	>11	<20
2		< 6	>16	
3	>12		>15	<20
4	> 6	<16	>16	<30
5	>12	<20	>30	<40
6		< 6	>24	<36

several hundred units of active material could be absorbed after oral ingestion without being detected by the means employed in these experiments. Consequently, the data presented in this report cannot be regarded as decisive evidence against the intestinal absorption of P.U., either in an unaltered form, or in the form of metabolically important fragments.

The results obtained from the specimens of *F.* and *W.* after the injection of more than 600 U of P.U. give the impression of utter inconsistency. Yet when these results are considered chronologically together with the symptoms produced by the succeeding injections, there appears a plausible explanation for the apparent inconsistency. The first intramuscular injection of P.U. (675 U) into these subjects produced general malaise, some headache and little else, the reaction being somewhat more marked in *W.* than in *F.* Three weeks later when the third injection (750 U) was made, *F.* experienced very little discomfort, but *W.* suffered a more marked reaction than he did in the first experiment. When the fourth injections were made (600 U) several months later, it was deemed advisable to perform skin tests on the subjects in view of the protein nature of P.U. and the history of allergy in *W.* The skin test was completely negative in *F.* but markedly positive in *W.*, the wheal lasting over 72 hours.[6] Despite a series of small, desensitizing doses, the injection of the remainder of the 600 U provoked an immediate reaction in *W.*, with weakness

[6] Subject W. does not react to the intradermal injection of phenol, so that the positive skin reaction to antuitrin cannot be attributed to the small amount of phenol used as a preservative.

and prostration, after which the subject was confined to bed with a fever of 101° F. The injection of the same amount of P.U. into *F.* at this time produced no symptoms whatsoever. Despite the fact that no skin test had been performed on *W.* before the first injection of P.U., the alteration in this subject's general reaction to these injections strongly suggests the development of a true hypersensitivity. Consequently one must consider the possibility that the repeated injections of P.U. led to some immunological change in these subjects. Viewed in the light of such possibility, one might explain the progressive decrease in the tannic acid yields, and the progressive increase in the average benzoic acid yields in those experiments in which 600 U or more was

TABLE 2. EXCRETION OF GONADOTROPIN FOLLOWING INTRAMUSCULAR INJECTION OF P.U.

P.U. Dose	Subject	Sample	Benzoic Acid		Tannic Acid	
675	W					
675	F	C1+2		<21	>148	<160
480	M	3		<43		<50
480	P	4		<65		<50
480	F	5		<60		<50
480	W	6		<60		<50
		C3−6		<25		<30
720	F	7			>38	<60
720	W	8			>38	<60
		C7+8	>34	<60		
600	F	9		<30		<30
	W	10	>60	<120		<30

† The experiments are listed in chronological order. Composite specimens are indicated by C. Assays are expressed in RB.U. per 24 hours.

injected intramuscularly, if the immunological change involved some modification in the form of the excretory product derived from P.U.

The postulation of more than one form of excretable gonadotropin is certainly justifiable when one considers the chemical and biological characteristics of the active materials in castrate urine, normal male urine, and pregnancy urine. The activity in pregnancy urine is effectively precipitated by benzoic acid, but this reagent is less effective than tannic acid in precipitating the activity from castrate urine (12). In this respect normal male urine resembles castrate urine rather than P.U. (compare benzoic acid and tannic acid yields in fig. 1 and table 1). Castrate urine is more potent in mice than in rats, while the reverse is true of pregnancy urine (13, 14). In this respect also male urine resembles castrate urine, as may be deduced from the data of Katzman and Doisy (p. 134). Such deduction is supported by the high values for the daily excretion of normal men obtained in the present experiments. These values can be reconciled with those of Katzman and Doisy (1 to 2 R.U.) only if we assume that the activity in normal male urine is much more effective in the rabbit than in the rat. This property of much greater potency in the rabbit is characteristic of castrate urine and anterior pituitary extracts (15, 16).

Although these biological and chemical differences may be presented as evidence for the existence of two or more gonatotropins, representing distinct products of internal secretion, the differences observed may easily be due to

some metabolic alteration incident to excretion. Likewise, the changes in the relative tannic acid and benzoic acid yields (table 2) during the course of the intramuscular injections might be attributed to an alteration of the manner of disposal of the injected material. Unfortunately, the individual specimens in these experiments were not all handled separately. Because of the differences in the reactions of the 2 subjects to the injections, and to the skin tests, this is particularly unfortunate. For obvious reasons, however, we are not eager to secure more data from these same subjects and therefore are content to submit the data from these experiments with the full realization that they permit no general statement regarding the fate in man of injected P.U.

SUMMARY

Benzoic acid determinations on the 24-hour urine specimens of normal males revealed a daily excretion of about 6 RB.U. of gonadotropic material. Tannic acid yields on urine specimens from the same subjects were consistently higher. From a consideration of available biological and chemical data, the gonadotropic substance of normal male urine appears to resemble the active material in castrate urine rather than the active material in pregnancy urine.

By the methods employed in these experiments it was impossible to detect any augmented gonadotropic excretion following the oral ingestion of 8,000 to 40,000 U of P.U. Similarily, it was impossible to detect any augmented gonadotropic excretion following the intramuscular injection of 480 R.U. of P.U. Repeated injections of larger doses of P.U. led to the excretion of variable fractions of the injected material, not exceeding 20 per cent of the injected activity.

The intramuscular injection of 480 to 750 R.U. of P.U. did not produce severe reactions in 3 of the 4 subjects. During the course of the injections 1 subject, with a history of allergy, developed an hypersensitivity to the pregnancy urine extract, as demonstrated by an intradermal test. This subject suffered an alarming reaction from an injection made several months after the first injections of P.U.

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OVARIES SECRETE MALE HORMONE

I. RESTORATION OF THE CASTRATE TYPE OF SEMINAL VESICLE AND PROSTATE GLANDS TO NORMAL BY GRAFTS OF OVARIES IN MICE[1]

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Grafts of ovaries have been used in many experiments as a means of studying the mechanism of ovarian endocrine function (1, 2). It has only recently been shown that intra-strain grafts in inbred mice yield as high a percentage of takes as when autografts are made (3, 4). The grafts described in this paper are all of the intra-strain type. The results of Hill and Gardner (5) open additional possibilities for the study of endocrine organs by grafting.

Ovaries grafted into the testes of mice continue to secrete estrogenic hormones (3). No great alteration of the prostates and seminal vesicles was described as a result of the intra-testicular grafts. On the other hand, the administration of large quantities of estrogenic hormone induced muscular and connective tissue hypertrophy as well as involution of the epithelium of the accessory glands[2] of normal male mice and rats (6, 7; Gardner, unpublished data). Injections of female hormones have never caused anything like normal development in the prostates or seminal vesicles of castrate mice or rats, i.e., the epithelium and its secretion have not been returned to normal and normal growth has not been obtained. Also, grafts of ovaries have never before produced results that could not be obtained by injections of female hormones.

The exocrine function of the testis is destroyed in cryptorchid animals. This has been shown to be due to the high temperatures maintained in the abdominal testis (8). The endocrine function of the testis disappears more slowly following the application of heat. The experiments described in this report deal with the function of ovaries which have been grafted into various sites of the host body. In so far as grafts in a cool medium (ear) come to have the endocrine function of testes, these experiments give rise to something of a counterpart to the experiments on cryptorchidism. More data and details are given here than in the preliminary report by Hill and Gardner (5).

MATERIAL AND METHODS

The animals used were sub-adult or adult mice, host and donor animals being of the same strain. A total of 8 non-related strains have been used so

[1] This investigation has been made possible by a grant from the Fluid Research Funds of Yale University School of Medicine. The author is indebted to Dr. W. U. Gardner for assistance in the experiments and for suggestions and criticisms.
[2] 'Accessory glands' in this paper refers to seminal vesicles and prostates.

far with comparable results. The technique of making ear grafts is very simple. A small skin incision is made in the dorsal side of the ear, near its base. A small pair of curved forceps are used to make a pouch under the skin, extending to near the tip of the ear. The ovary to be grafted is dissected free from excess tissues, and cut nearly in two in order to give a freshly cut surface

TABLE 1.

No. of animal Pg	Age at castration, days	Days castrated before graft	Days grafted when killed	Seminal Vesicles			
				Gross appearance	Epithelium	Secretion	Muscle
110	50	0	179	normal	normal	full	normal
127	36	54	152	"	"	"	"
185	46	0	139	"	"	"	"
199	52	0	92	"	"	"	"
200	52	0	92	"	"	"	"
202	45	0	252	"	"	"	"
228[1]	about 100	0	141	"	"	"	"
230[2]	" 100	0	141	"	"	"	"
104	38	0	194	½ normal	low-normal	slight	"
226[1]	about 100	0	Biop. 141	"	"	"	"
187	29	0	301	½ "	castrate	none	"
108	51	0	177	castrate	"	"	"
109	51	0	179	"	"	"	"
126	36	54	140	"	"	"	"
128	36	54	152	"	"	"	"
190	about 100	24	107	"	"	"	"
171[3]	37	0	190	"	"	"	"
172[3]	37	0	359	"	"	"	"
204[4]	56	0	245	"	"	"	"
205[4]	56	0	245	"	"	"	"

[1] Abdominal uterine and vaginal grafts (subcutaneous).
[2] Only 1 ovary grafted in addition to abdominal uterine and vaginal grafts.
[3] Abdominal uterine, vaginal and ovarian grafts (subcutaneous).
[4] Abdominal ovarian grafts (intraperitoneal).
In all animals except 171, 172, 204 and 205 the ovaries were grafted in the ear.

which aids in establishing a blood supply. The ovary is then pushed near the tip of the ear in the subcutaneous pocket. It is not necessary to fasten the graft in place or to suture the skin. Healing is rapid, and within 4 or 5 days an excellent visible blood supply has formed. Due to the thinness of a mouse ear the follicles and hemorrhagic areas of the graft can be easily seen. Growth is rapid, and the grafted ovaries soon become equal to or exceed the normal adult size.

EXPERIMENTAL RESULTS

Ear Grafts. So far 13 animals in which grafts of ovarian tissue have been present in the ears from 92 to 301 days have been autopsied (table 1). Some of these animals had been castrated for as long as 54 days before receiving grafts. Others were castrated at the time the grafts were made. Of these 13 animals, 6 carried normal seminal vesicles and prostates at the time they were killed, 2 had accessory glands of about one-half normal size, and the remaining 5 were of a typical castrate type. The combined seminal vesicle and prostate weight in 1 case, 200pg, was 150 mg., approximately 650 per cent larger than the castrate condition reported by Deansly and Parkes (9). The accessory organs from other animals, though equally large, were not weighed. Certainly the growth changes are most striking.

Study, by serial sections, of the accessory glands which had not developed grossly, showed that they were in a castrate condition. The epithelial lining was lower than normal and both the secretion and secretory granules were absent (fig. 1, *A*). The glands were tiny and histologically presented no actual hypertrophy of connective tissue and smooth muscle.

The secretion of the prostate, coagulating gland and seminal vesicles seemed normal in all positive cases. Rapid coagulation was obtained when the

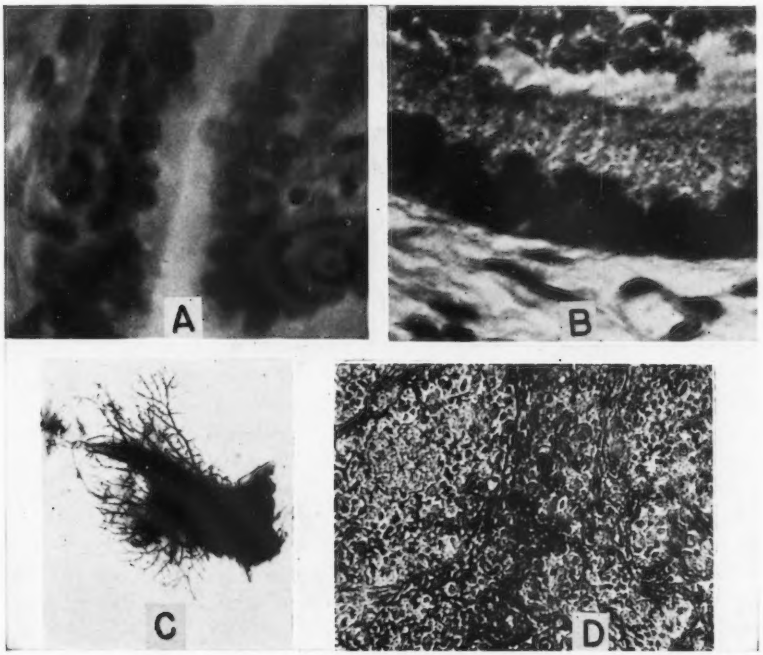

Fig. 1. *A*, Photomicrograph of seminal vesicle epithelium from 126pg. Complete absence of secretion in lumen and of secretory granules in epithelial cells. × 936. *B*, Epithelium from seminal vesicle of 127pg. Note granules in cells and secretion in the lumen. × 936. *C*, Whole mount of mammary gland from 127pg. × 3.2. *D*, Photomicrograph of the graft of ovarian tissue in the ear. × 149.

secretions were pressed from the glands. The epithelium was high and filled with secretory granules (fig. 1, *B*). The smooth muscle and connective tissue of these organs did not show hypertrophy as might be expected in the presence of ovarian or estrogenic hormones. At the same time we know that considerable estrogenic hormone was being secreted by the graft as shown in the mammary gland response (fig. 1, *C*).

A microscopic study of the grafted ovaries shows the presence, in most cases, of both normal and hemorrhagic follicles. The bulk of ovarian tissue appears as small clusters of lutein-like cells (fig. 1, *D*). Some bodies appear to be atretic corpora lutea. The medullary connective tissue of normal ovaries seems to have been obliterated. It cannot be said, at present, whether this

stroma has actually disappeared or whether it has been completely infiltrated and obscured by the above described lutein-like cells. Many cord-like arrangements of cells are present; however, they bear no semblance to embryonic testis cords. Indications are that these cords have proliferated from the germinal epithelium of the grafted ovaries, and that in their migration they have lost their identifying characters. Such cords are found in the grafts of only some animals with positive accessory gland growth, and also in some of the negative cases. At present we have no reason to connect any special cord arrangement of cells or other histological structure with the response elicited.

In one case, 185pg, laparotomy at 56 days after castration and grafting showed that the seminal vesicles were the size of those of a long-time castrate male. Yet, when the animal was killed at 139 days after grafting, the accessory glands were apparently normal. One hundred and eleven days after grafting an ovary in the ear of 202pg, the accessory glands were of a castrate type, although they were normal when the host was killed 141 days later. The shortest time in which a positive response has been obtained was 92 days (table 1). Further data are lacking as to the exact time element involved.

One isolated case may be mentioned here to good advantage. A female, 48pg, received grafts of ovaries in her ears when 1 month old. Her own ovaries were removed 221 days later, and 146 days after castration a piece of seminal vesicle from an immature male was grafted into one ear. She was killed 155 days after the graft of seminal vesicle was made. The vesicular graft was greatly distended with secretion. The epithelium appeared normal in some areas but not in others. None of the epithelium was a high as in normal male accessories, but the lack of height of the epithelium may easily be accounted for by the great distention of the glandular luminae. It appears that the state of development in this case, excepting the fact that the secretion had no escape, was quite normal. The animal was in heat when killed. These reactions were produced in a female by ovaries which had been grafted for 522 days. This part of the experiment is being checked in another group of animals.

Ovarian ear grafts combined with abdominal uterine and vaginal grafts. Three castrate males (266, 228 and 230; table 1) received grafts of ovaries in their ears at the time of castration, and at the same time the rest of the female genital tract (both uterine horns and vagina) were grafted under the skin of the mid-ventral abdominal wall. This type of grafting experiment was tried to see if the presense of the uterus and vagina would affect the response of the seminal vesicles. Two animals were killed at 141 days after the grafts were made and a biopsy of seminal vesicles was taken from the third. In 2 cases normal male accessories were obtained. In the third instance the seminal vesicles were about one-half normal size. The uterine grafts were large and distended with fluid and the vaginal grafts exhibited a completely cornified epithelium. The histology of the male accessory organs was normal in the 2 cases of complete development, and of near castrate type in the animal which carried the glands of about one-half size. These animals demonstrate, therefore, the absence of effect of uterine and vaginal grafts.

Grafts of ovaries on and in the abdomen. Two male mice each received 2 intraperitoneal ovarian grafts at castration, while 2 others each received subcutaneous abdominal grafts of ovaries, uteri and vagina (171, 172, 204, 205; table 1). These grafts took very well and functioned as did the intratesticular grafts described by Gardner (3). In the 2 animals carrying the complete female genital tract, the vaginae were patent to the outside. When vaginal smears were taken they contained only cornified cells. The histological picture supports this finding, and in substance it agrees with the observations of Hill (10) and Pfeiffer (11). In these 4 grafted males no sign of accessory sex gland development was evident.

Injections of ovarian hormones. Upon finding that accessory glands in the castrate male could be induced to grow and secrete due to the presence

TABLE 2. CASTRATE MALE MICE, RECEIVING INJECTIONS OF ESTROGENIC HORMONE AND PROGESTIN.

No. of animal Pg	Days injected	Total estrogenic hormone, I.U.	Total progestin, Allen-Corner RB.U.	Accessories
192	42	—	3.5	castrate
194	42	—	1.8	"
195	14	—	0.35	"
196	14	—	0.7	"
208	14	—	0.35	"
209	14	350	1.4	"
210	14	—	—	"
211	14	350	—	"
212	14	350	1.4	"
213	14	350	1.4	"
214	14	—	1.4	"

of grafted ovaries it was decided to see what could be done by injections of progestin, starting at different times after castration (table 2). In some cases the injections of progestin[3] were supplemented by the addition of estrin.[4] The greatest amount of progestin given was 3.5 RB. U. (Allen-Corner) during 42 days; the smallest amount was 0.7 RB. U. during 14 days. In 3 cases a total of 1.4 RB. U. of progestin were injected simultaneously with 350 I.U. of estroform 'B' in 14 days. Gardner (unpublished data) has injected castrate male mice with from 500 to 1000 I.U. of estrogenic material weekly up to 500 days, but has obtained no evidence of normal activation of the epithelium of the seminal vesicles or prostates. In the cases described here there was no gross change in the accessory glands observed following such injections. Atrophy of the accessories took place as rapidly during injections as in their absence. No growth resulted if atrophy was complete at the time injections were started. The length of the injection period and the quantity of hormone given did not effect castrate atrophy.

We must wait for future experiments to determine if female hormones (estrin and progestin) are capable of producing any of the effects normally brought about by male hormone.

[3] Progestin was kindly furnished by Upjohn Laboratories through the courtesy of Dr. Cartland.
[4] The estrogenic materials were kindly given to the author by Dr. A. S. Parkes.

Regional temperature of mice. It has long been known that temperature is a definite factor in the control of testis physiology. Realizing that temperature may eventually prove an important factor in explaining the peculiar ovarian physiology described here, it was decided that some normal regional temperatures should be presented (table 3).

A thermocouple, essentially like that described by Clark (12), was built which gave temperature recordings accurate to 0.1°C. The thermal junction was fastened in a hypodermic needle, but insulated from the needle. It can be introduced into any region of a mouse while under light anesthesia, thus giving the temperature of any desired point, independent from the surround-

TABLE 3. REGIONAL TEMPERATURES OF MICE, in °C.

No. of animal	Abdominal		Tip of ear	Tip of scrotum	Mid-tail
	Subcutaneous[1]	Intraperitoneal			
MSV 4	32.4	35.1	30.9	29.2	30.8
" 5	32.6	33.9	29.2	30.4	30.3
" 6	32.9	35.8	31.2	30.7	30.9
" 10	34.0	35.9	30.5	27.1	29.1
" 11	33.1	37.2	30.8	29.4	31.0
" 12	32.5	36.0	29.0	28.5	26.0
" 13	33.0	33.0	30.2	26.3	26.1
" 14	36.1	36.5	30.5	29.7	28.8
201pg	33.5	34.8	27.8	28.3	27.3
Average	33.3	35.4	30.0	28.9	28.9

[1] The subcutaneous temperatures were taken in a part of the skin which was held away from the muscles of the abdominal wall, otherwise they very closely approximate the intraperitoneal temperature.

ing thermal environment. The results, as given in table 3, are from a group of castrate male mice, each animal having one or more ovaries grafted in its ears. The ear temperatures given represent the immediate region in which the grafted gonad was growing.

Mice do not maintain as even a deep body temperature as might be expected, as variations are often noted which correspond with the surrounding temperature. It will be noticed that the temperature of the tip of the ear very closely approximates that of the tip of the scrotum and mid-tail, averaging from 29 to 30°C. These very specific regional temperatures are, on the average, from 5 to 6°C below that of the abdomen. As the temperature of the ear so closely approximates that of the scrotum, it seems that gonads in these two regions would be subjected to a very similar (cool) thermal environment. It is that similarity, coupled with the absence of secretion of male hormone by an ovary grafted in the warm abdomen, that suggests a possible temperature control of the output of male hormone from an ovary which has been grafted into the ear.

DISCUSSION

Although we have by no means exhausted the chances for possible effects of injected female hormones upon the castrate type of accessory glands in male mice, no detectable change was observed as a result of the injections here described. Also, no work has yet appeared in which female hormone

treatment has induced normal growth and secretion of the accessory organs of castrate males. Therefore, further consideration of this method of attack need not be given here.

The return of castrate seminal vesicles and prostates to normal as a result of an ovary grafted in the ear is a reaction which is not specific to a certain colony or strain of mice, for so far a total of 8 non-related strains have been used. No single one of these strains has failed completely to give positive accessory gland growth under the conditions of the experiment, although in 2 strains, using only 1 animal each, the response was somewhat less than in others. All of the mice used were from inbred strains, but it is doubtful if breeding is a factor in these experiments beyond its control of successful grafting. If there are any specific strain differences in the response of the seminal vesicles and prostates to ovarian ear grafts, then those differences as yet only point to some factor of time necessary to produce positive accessory gland development. It will be noted that 2 animals of one strain (I), 199 and 200, carried normal seminal vesicles at 92 days after the grafting of ovaries. Other strains required somewhat longer periods of time for the same reaction. Unpublished data makes it seem probable that, with other factors more adequately controlled, the differences in time of response between strains will prove of little significance.

This leaves only the last of the four possibilities mentioned by Hill and Gardner (5) to be considered; i.e., that male hormone may, in some cases, be "elaborated by an ovarian graft." This principle still seems to hold, and the suggestion is made that temperature may be the controlling factor. It is readily seen that the temperature of the ears of mice is much lower than abdominal temperature. Hence, any tissue which might be grafted into the ear is subjected to an entirely different thermal environment than if grafted in the region of the abdomen. Also, experiments on cryptorchidism have shown the effect of thermal changes on the physiology of the testis. In further support of the theory that temperature may enter into the reaction obtained is the fact that 4 experimental animals carried abdominal ovarian grafts (intra-peritoneal or subcutaneous) where the temperature was definitely above that of the ear. Although these grafts were carried for comparable periods of time (190 to 359 days) negative results were uniformly obtained. True, these results come from a small number of animals, but they are highly suggestive that the male hormone output was held in check by the higher temperature to which the grafts were subjected. Since injections of female hormones, as well as a careful histological study of the grafted tissues, have not as yet suggested a plausible explanation of the results obtained, a group of temperature control experiments has been started. It is hoped that such experiments will clarify the part that temperature may play in the secretion of male hormone by a grafted ovary.

SUMMARY

Grafts of ovaries in the ears of castrate male mice can cause normal growth and secretion of the seminal vesicles and prostate. Such growth and develop-

ment has never been approximated by female hormone injections. These ovarian grafts also secrete an estrogenic hormone as shown by the response of the rudimentary mammary glands of the male host mice. A similar reaction takes place in a female providing her ovaries are grafted into her ears and she carries a grafted seminal vesicle upon which the response may be registered. There is nothing in the histology of the graft which explains this peculiar physiology.

Abdominal ovarian grafts (intraperitoneal or subcutaneous) have not accomplished the same results as ovaries grafted in the ear. The reaction is not specific to any particular strain of mice.

Measurements of temperatures in mice show the ear approximates the temperature of the scrotum, both averaging 5 to 6°C below that of the abdomen. Therefore, the suggestion is made that temperature may be the controlling factor in the output of male hormone by an ovary grafted in the ear of castrate males.

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MALE HORMONE AND THE TESTIS-COMB RELATIONSHIP IN THE CHICK[1]

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Further study of the interaction of male hormone with the pituitary has been needed in order that the male reproductive physiology might be compared or contrasted more fully with that of the female. Early investigations (1, 2, 3, 4) demonstrated the inhibitory effect of estrin on ovarian weight in the rat, and an experiment suggestive of the same hormone relationship in the male has been reported (5) where a decrease in testicular weight of 12 to 50 per cent was noted in rats which had been given adrosterone. Recent descriptions of ovarian stimulation by estrogenic substances, especially under different conditions with respect to duration and amount of injection (6, 7, 8), however, do not have an adequate counterpart in experiments with the male. The fact that ample supplies of crystalline male hormone preparations are now available has removed one of the major difficulties which hampered investigation, and has provided additional stimulation for the study.[2]

EXPERIMENTAL PROCEDURE

The 270 chicks which constitute the material upon which this paper is based were all single-comb White Leghorns. Four male hormone preparations were injected: dihydroandrosterone-benzoate (Oreton-B); testosterone (Oreton); propionic ester of testosterone; and a male urine extract prepared in this laboratory. In addition, some series also received whole pituitary extract which was prepared by the pyridine method (9). All injections were made subcutaneously. The birds were sacrificed at intervals of 10, 15, and 30 days after hatching and body weights of all chicks were recorded. Statistical analysis of the data failed to show any significant correlations between body and gonad weight; and the body-weight figures accordingly are omitted from the tables.

RESULTS AND DISCUSSION

The variation in size of the daily dose given the different series was considerable, and ranged from 3 to 30 capon units of testosterone and 1.5 to 7.5 C.U. of Oreton-B. Despite this difference in dosage, the average testicular weight of the 67 birds which received testosterone and Oreton-B in the 10-day group showed a remarkable uniformity in range and average. Control and experimental results of the 10- and 15-day series are presented in table 1.

[1] Contribution No. 76 from the Waterman Institute, and No. 265 from the Zoology Department, Indiana University.

[2] The author expresses his appreciation of the courtesy of Dr. Gregory Stragnell and Dr. Max Gilbert of Schering Corporation for the supplies of crystalline male hormone used in these experiments.

These results show clearly that the injection of Oreton-B and testosterone in the chick under these experimental conditions was accompanied by almost immediate cessation of gonad growth and that this inhibition continued during the perod of injection. The ranges and averages for the 10-day experimental series showed a remarkable correlation with the 5-day and not the 10-day-old control chicks. Similarly, when injections were made from the 10th to the 14th day, as in series 81, the range of variation and the average for the series corresponded to that of the 10-day and not the 15-day control birds. Increase in the amount of hormone injected in the testosterone series was followed by increased inhibition. This was evidenced by the lower average weight and smaller variation in the range (series 46, 47, and 48). The small

TABLE 1. EARLY CONTROL AND MALE HORMONE SERIES

Treatment	Series	Age Days	No. of Animals	Gonad Weight*	
				Range	Average
Control	84	5	12	8.3–14.0	10.9
Testosterone					
0.1 mg., 5th to 9th	46	10	10	6.0–17.9	11.6
0.5 mg., 5th to 9th	47	10	10	7.4–15.8	10.5
1.0 mg., 5th to 9th	48	10	10	7.2–12.5	9.4
		Total	30		10.5
Oreton-B**					
0.1 mg., 5th to 9th	49+50	10	18	5.4–15.2	9.2
0.25 mg., 5th to 9th	80	10	9	8.0–15.8	11.7
0.5 mg., 5th to 9th	51	10	10	6.0–16.8	10.0
		Total	37		10.0
Control	28, 72, 83	10	25	9.2–29.5	17.9
Control	83[2]	15	13	19.5–50.5	30.0
Oreton-B					
0.25 mg., 5th to 9th	80[2]	15	11	7.6–28.9	20.5
0.25 mg., 10th to 14th	81	15	12	11.7–28.4	21.1

* Weight for the 2 testes in mg.
** Oreton-B (dihydroandrosterone-benzoate), all dosages indicated are amounts given daily.

number of animals does not eliminate the possibility that this result was coincidental, but the evidence seems very suggestive. The fact that the results with varied dosages in the Oreton-B series did not demonstrate the same phenomenon, however, somewhat complicates the picture.

Examination of the control weight averages in table 1 suggested that gonad growth from the 5th to 9th and 10th to 14th day might be commensurate and in approximately a straight line, thus implying a relatively constant amount of pituitary secretion in each period. Unfortunately, the inadequate number of control animals does not permit a satisfactory statistical analysis of this possibility. The experimental data, however, gave additional evidence in support of this uniformity.

It was shown that gonad growth stopped on the 5th day after the initial injection of Oreton-B and was held in check until the 10th day by subsequent injections. The period of growth in a 15-day experiment, therefore, would have been limited to the interval between the 10th and 15th days. Correspondingly, injection from the 10th to the 14th day would check growth during that

time and gonad increase would have been limited, by comparison, to the 5 to 10-day period. If the augmented gonad growth was regular for each 5-day period, as the control averages suggested, then the mean testicular weight in two such series should be equal on the 15th day, because each was inhibited by Oreton-B for 5 days and stimulated by its own pituitary secretion for 5 days. Experimental groups 80[2] and 81 had been injected in this manner, and the averages for the testes in the 2 series on the 15th day was 20.5 mg. and 21.1 mg. respectively. The experimental data, therefore, gave definite support to the hypothesis of commensurate pituitary secretion for each period. Attention has already been called to the similarity of series 81 to the 10-day controls. That series 80[2] also showed the same striking correlation is evidence that depression of pituitary secretion was temporary, lasting only through the injec-

TABLE 2. INJECTIONS OF WHOLE PITUITARY EXTRACT AND ORETON-B.[1]

Treatment	Series	Age Days	No. of Chicks	Gonad Weight	
				Range	Average
A.P. 5th to 9th day	77	10	9	15.5–42.6	28.6
A.P. and Oreton-B 5th to 9th day	79	10	10	14.5–43.7	25.4
A.P. 5th to 9th day / Oreton-B 10th to 14th day	76	15	14	13.0–64.6	28.5
Oreton-B 5th to 9th day / A.P. 10th to 14th day	82	15	9	28.3–67.4	43.0

[1] In all instances 0.1 gm. eq. of pituitary hormone was given daily and, where indicated, 0.25 mg. of Oreton-B daily.

tion period and apparently was followed by recovery within a short time.

Whole pituitary extract was injected in various combinations with Oreton-B in an attempt to analyze more fully the mechanism involved in the foregoing results. Oreton-B was injected simultaneously (series 79), after (series 76), and preceding (series 82) the injection of the pituitary hormone. These experiments are presented in table 2.

The pituitary-injected control series 77 showed about a 70 per cent net increment of gonad weight over the normal. Those birds which were given Oreton-B and pituitary hormone at the same time had an average gonad weight which was only slightly below that of the control series, 25.4 mg. compared with 28.6 mg. The range of variation again was remarkably alike in both groups, and it is doubtful if the difference in average weight was significant. The 28.5 mg. average for the testes of those chicks into which Oreton-B was injected following pituitary hormone, was almost identical with the control mean. Since these chicks were 5 days older than the controls, it is obvious that from the 10th to 15th day Oreton-B again exerted an inhibiting influence upon gonad growth. One bird which had gonads weighing 64.6 mg. was an exception and of course raised the mean in this series.

The fourth experiment, in which pituitary hormone followed the injection of Oreton-B for 5 days, was extremely interesting. The gonad weight at the time the pituitary hormone was first injected should have been about 10.5 mg.

(see 10-day Oreton-B series), or, since the birds for this series actually were taken from series 80 on the 10th day, testes should not have exceeded the average for that group, 11.7 mg. The pituitary hormone presumably would have been acting upon gonads which were like those of 5-day-old chicks. It was expected, therefore, that the weight at 15 days would correspond to that of the 10-day pituitary controls, 28.6 mg., and it was with some surprise that the average testicular weight of 43.0 mg. was noted. The lightest testes in this series equaled the control average and the heaviest represented the maximum size observed in the table. Because of the obvious relation of this series to the data to be presented in table 3, it will not be discussed further until after consideration of that material.

TABLE 3. MONTH-OLD CHICKS.

Treatment[1]		Series	Number	Gonad Weight	
				Range	Average
Control		45	10	28.4–77.5	52.7
		28	13	22.7–77.8	42.7
		Total	23		47.1
Oreton-B[2]	.1 mg.	67	10	41.7–132.2	76.0
	.25 mg.	68	8	23.4–137.4	73.4
Testosterone	.1 mg.	46+74	17	54.8–206.0	87.9
	.25 mg.	75	10	69.4–190.5	115.2
Propionic testosterone	.5 mg.	69+73	7	56.8–140.5	94.4
		Total	52		89.8
Male urine 50 cc. eq. daily		26	12	21.3–126.6	59.0
		Grand Total	64		86.5

[1] Experimental animals were injected for 5 days, from 5th to 9th day only.
[2] All amounts indicated represent daily dose.

The pituitary-Oreton-B combinations showed definitely that the action of the pituitary hormone was very slightly affected, if at all, by simultaneous injection of Oreton- B. Likewise, Oreton-B stopped gonad growth immediately when given following pituitary hormone injection. Finally, the adequate response to subsequent pituitary hormone treatment indicated that Oreton-B had done no fundamental damage to the testes which might prevent or partially inhibit response to subsequent pituitary hormone action. The results are in general agreement with the conclusions of other workers who described ovarian and testicular inhibition in mammals by estrone or androsterone injection and attributed this result to the failure of the pituitary gland to supply adequate amounts of gonadotropic hormone. The unexpected gonad growth which occurred when pituitary hormone injection followed Oreton-B and encountered in the 30-day chick experiments, confirm the more recent results (6 and 7) which demonstrated ovarian stimulation after injection of estrogenic hormones. This can be explained best after reference to the figures for the older birds given in table 3.

The gonad average of the two 30-day control series was somewhat more variable than might be desired; but the ranges, as was the case in nearly all these data, were again similar. Unexpected results were encountered when

the male hormone series were analyzed. After the injection of the synthetic preparations, the average testicular weight of 89.8 mg. showed a net increase of 90 per cent over the control. The 52 animals in the group were ample in number to make the results significant. With the exception of series 68 and 26, minimum gonad weights in the experimental groups were decidedly higher than the minimum for the controls and the maximum weight in all series was

TABLE 4. COMB GROWTH IN 10 AND 30 DAY SERIES. COMB RATIO = $\sqrt{H \times L}$ MM.

				10-Day Series					
Control 83									
Comb Ratio	5.5	5.2	4.2	5.0	4.8	4.8			
Gonad Weight Av.	28.0	23.3	22.0	18.3	15.8	11.5			
Oreton-B 80									
Comb Ratio	10.0	9.1	9.0	7.7					
Gonad Weight Av.	15.7	13.5	10.5	8.0					
Pituitary Extract 77									
Comb Ratio	10.0	9.0	8.4	7.0	5.5				
Gonad Weight Av.	42.4	29.4	27.0	22.0	15.5				
A.P.+Oreton-B 79									
Comb Ratio	9.4	8.5	8.0	8.0	7.1				
Gonad Weight Av.	43.7	32.7	29.4	22.1	16.4				
				30-Day Series					
Control 28									
Comb Ratio	18.9	13.3	19.0	13.6	12.3	11.0	9.9	9.2	
Gonad Weight Av.	77.8	65.1	51.9	48.2	38.7	32.6	25.9	22.7	
Oreton-B 67									
Comb Ratio	37.0	34.2	26.0	26.2	26.7	25.6	24.6	23.6	23.2
Gonad Weight Av.	132.2	109.2	105.0	92.6	67.7	78.3	52.4	45.3	41.7
Oreton-B 68									
Comb Ratio	34.1	31.4	30.3	30.7	30.0	26.7	27.2	20.5	
Gonad Weight Av.	137.4	101.4	94.2	71.4	84.4	41.2	34.1	23.4	
Testosterone 74									
Comb Ratio	33.3	37.0	35.3	28.3	29.0	28.0	18.0	32.8	
Gonad Weight Av.	206.0	127.4	119.0	104.0	103.5	95.8	72.5	54.8	

greatly in excess of that in the controls. The heaviest testis, 206 mg., represented a net increment over the maximum of the controls of nearly 165 per cent. The smallest average increase in the entire group was found in the male urine injected series, but it should be noted that a total of only 250 cc. equivalent of the original urine was given; thus the amount of hormone injected was small.

It has been reported that injection of androsterone into the rat (5) was followed by testicular injury and loss of gonadotropic activity of the pituitaries. It is plain, under the experimental conditions employed in the chick, that neither observation applied here, except perhaps temporarily. On the contrary, it was evident that a mechanism developed which resulted in an augmentation of gonad growth. Ample evidence that such an increase existed is indicated in table 3, and by the experiment in which pituitary hormone was injected following 5 days of Oreton-B treatment. There are, at least, two logical explanations, either the pituitary was stimulated to greater secretion, or the

gonads were more responsive to the same given amount of hormone. Comparison of series 80[2] with 81 is inconclusive on this point as the time interval was evidently too short to permit a significant expression of increased testicular response. Likewise, the results in the immature female rat do not clarify the problem because, while the ovaries of the hypophysectomized rat did not undergo hypertrophy following injection, this merely demonstrated that no response was elicited in the absence of pituitary hormone.

The increase in the size of other endocrine glands observed (6) when the intact rat was injected, would lend support to the idea of an increased rate of pituitary secretion. It would hardly be expected that a 'priming' effect would have been exerted on the entire endocrine system. It seems exceedingly probable that both factors were involved in the response of the chick testes, since a direct effect of estrone on the ovary, in the apparent nature of a synergist, as well as a stimulation of the pituitary has been described in the rat (8). Adequate analysis of the augmentation phenomenon must await further experimental study, perhaps with hypophysectomized fowl.

The marked response of the comb of the immature chick to male hormone is known (10). Nevertheless, in view of the contrasting picture of testicular inhibition followed by stimulation, in these series, it was considered important to check carefully the relationship between comb and gonad size. The usual dilemma was encountered as regards the proper method of establishing a factor for comb size. After rather extensive measurement of a few combs, the figure represented by the square root of the height times the length measured in millimeters, appeared to be the most practical. This comb factor, of course, represents only two dimensions. Undue weight accordingly was given the control birds when comb comparisons were made because volume development of these was in some cases less than one-fifth that of the experimental chicks.

Several typical series are presented in table 4, in which comb size and gonad weights are given. A few figures represent averages which were made when both comb size and gonad weights were nearly equal.

A definite correlation between comb size and gonad weight appeared within each series in both the 10 and 30-day control animals, but not between series. Only one marked exception to this was present in the 30-day controls, the 51.9 mg. tests had a comb factor which was larger than the average. Two experimental birds discussed later also were not in harmony with the other results.

Increase in size, turgidity, and hyperemia of the comb became apparent in the Oreton-B series within 60 hours after the first injection, and by the end of the 5th day the largest combs exceeded many of those found in the 30-day control animals. This was especially evident if the third dimension of the comb was considered.

Injection of pituitary extract was followed by definite comb growth within 72 hours, and combs in this series compared favorably with those of the male hormone group. The gonad-weight range in the pituitary series coincided in

part with that of the control, but it was of interest that the combs of pituitary-injected birds exceeded by a significant amount those of chicks in the control series with similar gonad weight. For example, in the pituitary series the 22.0 mg. gonads were accompanied by a comb factor of 7.0; the 29.4 mg., 9.0; but in the control animals 22.0 mg. and 28.0 mg. testes were matched with combs which had factors of 4.2 and 5.5. This indicated that weight of the gonad, per se, did not determine the amount of male hormone which was secreted and checks with observations made previously (11) that secretion of male hormone, measured by comb size in birds given pituitary hormone, was correlated with development of the seminiferous tubules, but was not comparable merely with an increase in testicular weight (12).

The combs of the injected chicks continued to grow at a remarkable rate after the end of the 10th day. Comb factors of 30 or above appeared 11 times in table 3 with a maximum of 37 appearing twice. One bird (not shown) which had to be killed because of injury when 2 months old, had a comb which measured 52 mm. x 83 mm., a factor of 65.6, and was large enough to cause the chick considerable inconvenience. The data showed that there was a tendency for comb growth to be about uniform in those chicks having gonads of about 60 to 100 mg. More extensive analysis is necessary to determine if this plateau was of real significance.

There were 2 exceptional comb-gonad relationships in the 30-day series. One chick with gonads which weighed 206 mg. had a comb which was smaller than that of a bird in the same series with 127.4 mg. testes. The second animal having gonads of 54.8 mg. had a comb larger than that of chicks which averaged slightly over 100 mg. and peculiarly enough, was nearly as large as the comb of the animal with 206 mg. testes. These observations confirm the conclusion that while comb size tends to follow testicular weight, there is also a differential factor involved which, it is suggested, may be referable to the degree of tubular development in the gonad.

In conclusion, the most suggestive correlations appeared when the ratio of the maximum and minimum comb growth of the male hormone and control series were compared for the 10 and 30-day groups. The ratio of the Oreton-B maximum to the control maximum in the 10-day experiments was 1.8 to 1, the minimum 1.7 to 1. The ratio in the 30-day group was 1.9 to 1 for the maximum and 1.9 to 1 for the minimum. The gonad ratios on the other had changed from 1 to 1.7, favoring the controls in the 10-day chicks to a ratio of 1.9 to 1 in favor of the experimental birds in the 30-day series.

The very close resemblance of the ratios for comb size in two separate time series, under different hormone levels, implies that there may be a definite limit to which comb growth can be pushed. This is also supported by a comparison of series 79, which was given both Oreton-B and pituitary hormone simultaneously, with series 77 which received only pituitary extract. The comb sizes in the former did not exceed those of series given pituitary hormone alone, except in the lower gonad-weight ranges. Furthermore, the data signified that the intact chick behaved, with respect to comb response, essentially

as though caponized. It is obvious that the early stimulus to comb growth was supplied by male hormone injection but was subsequently maintained in the same ratio (1.9 to 1) by secretion of the animals' own gonads.

SUMMARY

Male hormone preparations, dihydroandrosterone-benzoate (Oreton-B) and testosterone (Oreton) injected for 5 days into 5 or 10-day-old chicks caused immediate cessation of testicular growth which apparently lasted only during the period of injection. Month-old chicks, however, which had been treated with male hormone preparations from the 5th to 10th days of age had an average gonad weight 90 per cent heavier than the control. This was in harmony with the augmented testicular response observed when pituitary hormone followed Oreton-B in the shorter 10-day experiments. Combination injections with pituitary hormone and Oreton-B showed that the latter acted indirectly by suppressing the secretion or release of hormone from the animals' own pituitary.

Ratio of comb growth in Oreton-B treated chicks to that of the controls at 10 days was 1.8 to 1. The ratio at 30 days was still favorable to the experimental series by 1.9 to 1. Amount of comb growth tended to be correlated with gonad weight. Some exceptions were noted and the possibility of difference in male hormone secretion due to differential development of the seminiferous tubules was advanced as a possible explanation.

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REVERSAL OF ESTRIN-INDUCED PROSTATIC PATHOLOGY IN MICE BY THE USE OF TESTOSTERONE[1]

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Recent work indicates that a sex hormone imbalance is responsible for prostatic pathology. This becomes evident when the male hormone is decreased in relation to the estrogenic hormone. Thus, in men afflicted with prostatic hypertrophy, such a deviation from the normal ratio has been reported (1). Furthermore, an imbalance may be induced in male mice, rats, dogs and monkeys by the administration of estrin, producing changes in the prostate gland which, in some respects, are similar to prostatic enlargement of men. MacCallum (2) and Zuckerman (3) have recently summarized this relationship of the sex hormones to prostatic pathology. An attempt to inhibit the prostatic changes, artificially induced in mice, by restoring the normal hormonal balance, would appear worthy of trial. Such efforts have been made.

Burrows (4) reports that progesterone protects normal male mice from the keritinizing effect of estrin on the prostate. This did not hold true in castrated mice. He concludes, then, that the male hormone acted synergistically with progesterone to prevent these changes. DeJongh (5) states that an injection of an equivalent amount of male hormone protects the prostate of mice, but the corpus luteum hormone gives no protection. Weller, Overholser and Nelson (6) found that male hormone and antuitrin-S given simultaneously with estrin in mice prevented the metaplasia of prostate epithelium but did not reduce the bladder dilatation. Korenchevsky and Dennison (7) noted that male hormone administered simultaneously with estrin in castrated rats almost completely prevented the pathological changes induced by estrin. Overholser and Nelson (8), however, state that estrin and the male hormone act synergistically upon both smooth muscle and epithelium of the rat prostate when given at the same time. Zuckerman and Parkes (9) found androstanediol not only suppressed the prostatic effects of estrin in monkeys when given in the proportion of 50:1 but in one experiment they demonstrated that after the pathology had been produced, androstanediol restored the prostate toward normal. Progesterone, in the doses used, had no effect. Zuckerman (10) also reports that testosterone propionate inhibits the effect of estrin on the prostate and urethra of monkeys when the amount of the hormone is 7 times greater than that of estrin.

In the experiments cited, with the exception of one in which only one monkey was used (9), the 'inhibitory' substances were administered simultaneously with estrin from the start of the experiment. Information thus gained is of value in determining whether or not such substances are capable of inhibiting the effects of estrin. It is the purpose of this report to determine

[1] This study was supported by the Jonathan Bowman Grant for cancer research.

whether a reversal of the changes induced by estrin in the prostate of mice can be elicited by the administration of 'inhibitory' hormones after the pathology has been established.

PROCEDURE

Male mice of a pure albino strain aged from 6 to 8 weeks were used. Subcutaneous injections of from 5 to 50 γ of estrin in oil (progynon-B[2]) were given twice weekly for periods of either 122 or 137 days. Pathological changes were present after a period varying from 30 to 70 days at which time subcutaneous injections of testosterone (oreton[2] 125-250 γ); progesterone[3] (250-1250 γ), or dehydroandrosterone[3] (1250-2500 γ) were started and continued twice weekly simultaneously with estrin for the remainder of the experiment. Five control groups were maintained; one group received only estrin, another

No. of mice	Estrogenic hormone	Dose twice a week γ	Total γ	Total days given	Other hormone	Dose twice a week γ	Total γ	Day[1] started	Total days given	Wt. of prostate[2] mg.	Metaplasia of glandular Epithelium
4	Progynon-B	25	875	122	Testosterone	125	1,750	70	52	70	±
2	"	25	875	122	"	125	4,375	1	122	82	±
4	"	25	875	122	"	250	3,500	70	52	122	0
2	"	25	875	122	"	250	8,750	1	122	92	0
2	None	—	—	—	"	125	4,375	1	122	154	0
2	"	—	—	—	"	250	8,750	1	122	178	0
4	Progynon-B	25	875	122	None	—	—	—	—	31	+
3	"	5	190	137	"	—	—	—	—	36	+
2	"	5	190	137	Progesterone	250	2,000	107	30	28	+
2	"	5	190	137	"	500	4,000	107	30	35	+
5	"	12.5	475	137	None	—	—	—	—	26	+
2	"	12.5	475	137	Progesterone	500	10,000	67	70	36	+
3	"	12.5	475	137	Dehydro-androsterone	1250	20,000	76	61	40	+
4	"	25	950	137	None	—	—	—	—	35	+
2	"	25	950	137	Progesterone	1250	23,750	70	67	49	Degeneration
1	"	25	950	137	Dehydro-androsterone	1250	31,250	44	93	31	+
1	"	25	950	137	"	2500	62,500	44	93	29	+
2	"	50	1900	137	"	2500	12,500	120	17	31	+
2	"	50	1900	137	Progesterone	1000	7,000	112	25	35	+
5	None	—	—	—	None	—	—	—	—	184	0
5[3]	"	—	—	—	"	—	—	—	—	33	0

[1] The number of days that the mice had been receiving progynon-B prior to the time when the second hormone was started. The second hormone was then given simultaneously with the progynon-B for the remainder of the experiment.
[2] The combined weight of both seminal vesicles and prostate per 25 gm. of total mouse weight.
[3] These mice were castrated for 15 days prior to time they were killed.

got only testosterone, a third group were given both of these hormones throughout the course of the experiment, a fourth group of normals received no injections, and a fifth group of castrates were not injected. Exact dosage and the day when each hormone was started is given in the accompanying table. At the end of this time, the mice were killed and weighed. The prostate and seminal vesicles carefully removed in one piece, weighed, and sectioned for microscopic study.

In order to understand the results better, a brief summary is given of the pathological changes induced in mice by the administration of estrin. Histological examination of the prostate reveals that the epithelium passes through definite stages: *a)* an arrest of function; *b)* hyperplasia; *c)* metaplasia into a

[2] Progynon-B, the benzoic-acid ester of dihydro-estrin and oreton, synthetic testosterone in oil were obtained from the Schering Corporation through the courtesy of Dr. Max Gilbert.
[3] Progesterone, free from estrin, was extracted from sow ovaries by the method of Allen and Meyer (11). Dehydroandrosterone was a synthetic product prepared as described by Wallis and Fernholtz (12). I am indebted to Dr. M. A. Spielman of the Chemistry Department of the University of Wisconsin for these preparations.

stratified squamous epithelium (13). An increase in the fibromuscular stroma of the prostate occurs, but in spite of this, the size and weight of the prostate and seminal vesicles decreases to approximately the same size as those present in castrated mice. Shortly after these changes are present, the urinary bladder becomes dilated and scrotal hernias develop.

Testosterone induced a reversal of the stratified squamous epithelium to the normal columnar type, and it produced increased secretion of the epithelium as was evidenced histologically as well as by a weight increase of the seminal vesicles and prostate. Such changes were somewhat proportional to the dose given. Thus, 125 γ of testosterone twice weekly did not completely abolish the metaplasia as was evident by the fact that some layering of cells remained, although the cells were beginning to resemble those of a columnar type. With this dose of testosterone, the average combined weight of the prostate and seminal vesicles was 70 mg. as compared to 31 mg. for those receiving only estrin. Injections of 250 γ of testosterone given twice a week increased the average weight to 112 mg. and completely abolished the metaplasia. The average weight of the prostate and seminal vesicles of mice castrated for 15 days was 33 mg.

Testosterone had little or no effect in reducing the amount of fibromuscular stroma. It did not reduce the size of the dilated bladders nor the scrotal hernias, although when started from the beginning of the experiment, it delayed the appearance of these conditions for 40 to 50 days.

In the doses used progesterone as well as dehydroandrosterone did not induce a reversal of the metaplasia or cause a weight increase of the prostate as did testosterone. In a mouse in which a total of 23,750 γ of progesterone was given, the epithelium of the prostate which had undergone metaplasia, appeared to be degenerating.

DISCUSSION

Since the literature contains references to the pathological changes produced in the prostate of mice by estrin as a prostatic hypertrophy although there is actually a weight decrease, some confusion results. To avoid such confusion, one must distinguish between an increase in size and weight of the whole gland and the hypertrophy only of certain tissues. In the mouse, a large part of the size of the prostate is the result of the glands being distended with secretion. The fibromuscular stroma is responsible for only a small percentage of the weight. Thus, if a substance is given which arrests secretion, a loss of weight and size of the gland becomes evident in spite of an increase in fibromuscular stroma. The changes induced in the prostate of other animals by estrin are similar to those described in the mouse, but because of a different proportion of fibromuscular stroma to glandular epithelium, the end results, so far as weight and size are concerned, vary in different species.

It also appears that the degree of hyperplasia of the glandular epithelium induced by estrin varies in different species. In dogs and monkeys, it is more marked than in mice (9). Thus in dogs, where an increased proportion of fibromuscular stroma is also present, an increase in size and weight of the prostate occurs following the administration of estrin.

Estrin given over a long period of time to mice seems to manifest itself by both a direct and an indirect effect. It probably inhibits the production or liberation of the gonadotropic hormone and thus through the lack of gonadal stimulation there is an arrest of glandular secretion of the prostate. The male hormone appears to be responsible for the physiological integrity of this gland (14). The metaplasma of the epithelium, the increase in fibromuscular stroma, and the bladder dilatation and scrotal hernias are probably the result of a direct action. At least, these changes have not been described following the injection of other hormones or after castration or hypophysectomy.

Zuckerman (10) reports that in monkeys the prostatic changes produced by estrin are inhibited by testosterone propionate if the latter is 7 times the amount of estrin by weight. When considering only the amount of estrin given during the period in which the 'inhibitors' were also administered, it is noted from these data that a partial reversal of the metaplasia was induced when 5 times more testosterone than estrin was administered, and brought about a complete reversal when 10 times more of testosterone was given. Progesterone induced a degeneration of the epithelium when 50 times the weight of estrin. Ninety-six times more dehydroandrosterone than estrin by weight did not restore the prostate toward normal.

The theory that the sex hormones in animals and man are in a balance, the deviation from which may result in pathology, appear to be corroborated by recent work. In animals this balance may be upset by the injection of an estrogenic hormone with resulting pathology. In man spontaneous prostatic hypertrophy is believed by some to be the result of a sex hormone imbalance (3, 15, 16). Recent studies by Rusch and Kundert (1) in which it was shown that the male hormone is decreased in proportion to the estrogenic hormone in men with enlargement of the prostate, strengthens such theory. If such a deviation from a normal balance of sex hormones is responsible for hypertrophy of the prostate, then since certain changes produced by excess estrin in mice may be partially corrected by administering testosterone, the trial of replacement therapy in man for the relief of prostatic hypertrophy appears to be justified. This may be attempted either by the administration of a male hormone or by the stimulation of the testes with gonadotropic hormones. Zuckerman and Greene (17) and Niehans (16) have reported some encouraging results with such therapy.

The problem, however, is probably not so simple. Different hormones other than those used in the experiments described may probably be involved. Since testosterone inhibits only certain changes produced by estrin and not others, at least in the doses used, its theoretical value as a therapeutic agent in prostatic hypertrophy is only partially justified. Furthermore, the pathological changes produced in the prostates of animals is brought about only by the administration of estrin in amounts larger than would normally be present in the body.

SUMMARY

Long period injections of estrin into male mice induce changes in the prostate gland characterized by a metaplasia of the glandular epithelium into a

stratified squamous type, an increase in the fibromuscular stroma, and a weight decrease of the whole gland. Testosterone, when administered simultaneously with estrin, after these pathological changes had been established, induced a reversal of the metaplasia toward a normal type and was responsible for a weight increase of the gland, but had little effect upon the fibromuscular stroma. Progesterone and dehydroandrosterone, in the doses used, had no such effect.

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THE USE OF GLYCEROL EXTRACT OF THE ADRENAL CORTEX IN THE TREATMENT OF ADRENAL INSUFFICIENCY[1]

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It appears that Obrigia (1) was the first to use a glycerol extract of the adrenal gland. This was employed in the treatment of patients with hyperthyroidism. Later Rogoff and Stewart (2) employed glycerol extracts in the treatment of patients with Addison's disease. Rogoff and Stewart extracted the cortex after removing the medulla but made no further attempt to reduce the epinephrin content of their extract. Such reduction is desirable because epinephrin is detrimental in some patients. (The cortex contains about 1/5 as much epinephrin as the medulla, per gm.). For 5 years the authors have used a glycerol extract of the adrenal cortex, from which much of the epinephrin had been removed. Patients with Addison's disease and some of those with suspected adrenal insufficiency have responded favorably to such treatment.

Fresh, ground beef cortex is extracted with an equal volume of glycerol at 4° C. for several days. Then, an equal volume of alcohol is added to reduce the viscosity so that it can be passed through 'crystallite.' This removes epinephrin and other substances which may be toxic. The alcohol is distilled off in vacuo. The amount of epinephrin remaining in the extract ranges from 1:10,000 to 1:50,000 as determined by the intestinal strip method. One cc. of the final extract contains the product of approximately 1.5 gm. of cortex. Great care must be taken in the preparation of the extract since cortin is easily destroyed by heat and by alkali (3).

It is difficult to assay the cortin content of glycerol extract. This was attempted in 2 bilaterally adrenalectomized cats whose cortin requirements had been established. The glycerol extract was mixed with their food. It was possible to substitute glycerol extract for 35 to 50 per cent of the required injected cortin. The refusal of the animals to ingest larger amounts of extract limited the substitution.

Glycerol extract offers certain advantages. The simpler method of preparation makes it less expensive than the purified aqueous solution. Glycerol is a good preservative as well as a good solvent for cortin. It is also possible that glycerol gives a measure of protection to the hormone in the alimentary canal thus making oral administration feasible. The amount of cortical extract which may be administered in this way is limited by the patient's tolerance for glycerol. The addition of a flavor such as oil of wintergreen renders the prepa-

[1] Aided by grants from the Carnegie Foundation and the Rockefeller Foundation.

ration more palatable. This extract is best administered *post cibum* and should be followed immediately by water. The usual dosage employed has been from 2 to 4 cc. 3 times daily. Occasionally as much as 20 cc. per day has been administered.

In 9 patients with severe Addison's disease an attempt was made to substitute glycerol extract for cortin injections. In all of the patients the severity of the disease was such that weight loss, dehydration and a relapse followed the withdrawal of cortin. In 6 of these patients the effect of ingesting large amounts of sodium salts was studied without cortin. Ten to 15 gm. of added sodium chloride or a mixture of sodium chloride and sodium bicarbonate were not sufficient to prevent a relapse.

The 3 cases herein presented are typical of the group with a severe stage of the disease. Case 1, has been treated successfully with glycerol extract for a long period of time. She is able to carry on normal activities with continuous treatment. Case 2, did well as long as treatment with glycerol extract was continuous. He felt so well that he took the liberty to discontinue treatment, while he was traveling. The more strenuous duties together with the absence of treatment brought a relapse. Since then injected cortin has become necessary. Case 3, was progressing so well that she was allowed to return home but the treatment with glycerol extract was not sufficient to combat the results of trauma and pneumonia.

These patients illustrate the limitation of glycerol extract therapy in severe Addison's disease. Its use in Addison's disease should be attempted only when the patient's hormone requirement is small.

Case 1. Patient A.Z., a white female, 43 years of age, was examined Dec. 31, 1934. Since 1930 there had been present, increasing muscular weakness, nervous irritability, sensitivity to cold, muscular pains, weight loss and a generalized bronzing of the skin. Early in 1917 the patient developed symptoms of tuberculosis following which she was hospitalized for 6 months. She was discharged as an arrested case of pulmonary tuberculosis. Although the patient gained 26 lb. in weight she never returned to full physical vigor. Since 1934 the patient had been a semi-invalid. In 1934 a diagnosis of Addison's disease was made by her physician and treatment with injected cortical extract was instituted.

The patient was poorly nourished, weighing 46.3 kg. Her skin was dark and dry with a generalized increase in pigmentation. The cardiac impulse was feeble. The heart rate was 78 per minute in the recumbent position and 96 per minute while sitting. A soft blowing murmur was heard best over the pulmonic area. The blood pressure was 70 mm. Hg systolic, 56 mm. diastolic. The entire muscular system showed a moderate degree of wasting. Muscle tests revealed marked fatigability. Ankle clonus and Babinski's sign were present on the left side only.

Blood examination showed a slight secondary anemia. The hemoglobin content was 87 per cent. There was an increased tolerance to glucose. The basal metabolic rate was —7.7 per cent. Following the administration of alcohol the free hydrochloric acid in the gastric analysis amounted to 39 per cent and the combined acid to 9 per cent.

The patient was given 4 to 6 cc. of glycerol extract per day. When treatment was begun the patient was a semi-invalid. During the past year she has been able to carry

on her housework and to take long walks. Her blood pressure has increased to 100 mm. Hg systolic and 58 mm. diastolic. The patient sleeps better, her appetite has improved and she has gained 4.54 kg. in weight. There is a decided decrease in the amount of pigmentation. The ankle clonus has disappeared. The improvement in the muscular system was demonstrated on the ergometer. The results were as follows: Feb. 2, 1935, 36,000 gm. cm. work; Feb. 24, 1935 (after treatment) 108,000 gm. cm. work; April 27, 1936, 450,000 gm. cm. work Withdrawal of the extract results in a return of symptoms and clonus. The patient has not found added sodium chloride especially beneficial. She has been treated successfully with glycerol extract for 2 years.

Case 2. Patient A.L., a white male teacher, 40 years of age, was examined on March 15, 1935. Since Sept. 1934 the patient had complained of weakness, loss of appetite and weight, increased nervous irritability and a peculiar discoloration of the skin (vitiligo). This condition culminated in severe prostration which was termed an attack of influenza. Recovery was delayed. During December 1934 and January 1935 symptoms of increased gastro-intestinal irritability was associated with nausea, vomiting and weight loss (13.6 kg.). The blood pressure was 80 mm. Hg systolic and 50 mm. diastolic. In 1927 the patient had had pneumonia complicated by pleurisy. A diagnosis of pulmonary tuberculosis was made at that time although subsequent examinations failed to confirm this.

The physical examination showed evidence of marked weight loss. The pulse rate was 85 per minute and the blood pressure 80 mm. Hg systolic and 50 mm. diastolic. Areas of vitiligo surrounded by deep pigmentation were distributed generally over the body. Aside from the general asthenia there were no other abnormal physical findings.

The laboratory findings disclosed a marked secondary anemia. A diagnosis of Addison's disease was made and treatment with glycerol extract was begun in March 1935. Five days after starting the extract the patient noticed reduced fatigability, decreased nervous irritability and lessened gastro-intestinal distress. With continued treatment the patient's blood pressure increased, his appetite improved and he gained weight. Added sodium chloride when combined with extract resulted in further improvement. During June and July 1935 the patient was maintained on sodium chloride alone. During June the patient did well but during July he failed to gain weight and his nervous irritability returned. At this time he was given glycerol extract with a good response. During the month of September the patient traveled considerably. At this time he discontinued both extract and salt. One week later he began to fatigue more readily. Three weeks later his appetite began to fail and in October 1935 he became so ill that it was necessary to inject cortin. At this time the patient was admitted to the University Hospital where electrolyte balance studies demonstrated a retention of sodium and chloride during the period of cortin injections.

Cortin injections (6 cc.[2] per day) supplemented by sodium chloride (8 gm. per day) have been continued for 15 months. His condition has improved so that he is able to carry on his teaching. On June 10, 1936 his blood pressure was 104 mm. Hg systolic and 70 mm. diastolic. The pulse rate was 84 per minute. It is possible that if treatment with glycerol extract and sodium chloride had been maintained, the need for injected extract could have been prevented or at least postponed.

Case 3. Patient R.M., a white female, 24 years of age, single, was examined Oct.

[2] 1 cc. of the extract contained 4 C.U. of cortin.

12, 1936. This patient was in excellent health and working 10 to 12 hours a day until July 1935. At this time she spent a period of 3 weeks at a summer resort where she was exposed to the sun several hours each day. She acquired a deep tan which did not disappear in the autumn. In November the coat of tan actually became darker especially on the backs of the hands and face. During the winter dark pigmented spots, pin head in size, appeared on the exposed parts of the hands and forearms. Deep purplish pigmentation of the gingival margins, lips, dorsum of the tongue and buccal surfaces of the cheeks appeared. At the same time she began to complain of unusual fatigability. All signs and symptoms increased in intensity until May 1936 when it was necessary for her to reduce her working hours. The condition was then diagnosed as Addison's disease and irregular treatment with adreno-cortical extract instituted. The injections which were first given at weekly intervals were followed by temporary relief from fatigue. In September she was advised to discontinue working. The enforced rest seemed beneficial. There were neither digestive disturbances nor weight loss. There was some indefinite pain in the arms. The hair which was very dark had recently become brownish and dull.

The patient was a well-developed, well-nourished white female apparently in good health except for the pigmentation as described and a tendency to be nervous when questioned. The pulse rate was 100 per minute, regular and soft. The blood pressure was 90 mm. Hg systolic and 70 mm. diastolic. The laboratory findings were essentially negative except that the CO_2 combining power of the blood was 49 vol. per cent.

Oral administration of glycerol cortical extract was begun Oct. 26 with 2 cc. glycerol extract 3 times a day. On Nov. 11, the dosage was increased to 4 cc. 3 times a day and Dec. 1, 6 gm. of sodium chloride per day was added. During her stay in the hospital there was considerable improvement in every respect except the blood pressure did not change. The pigmentation faded, nervousness decreased and the appetite showed remarkable improvement. The patient slept well and said she felt better than at any time in the past 18 months. The CO_2 combining power of the blood rose to 57 vol. per cent on Dec. 7. There was some increase in blood volume, which study was discontinued because of severe reaction to injection of the dye.

On Dec. 8, the patient was allowed to return home about 100 miles from the hospital to continue the oral treatment and with the warning to refrain from much activity. She continued treatment but engaged in strenuous preparation for the Christmas season. It was noticed by her family and friends that after one week at home, she was very nervous, active and losing strength. This may have been responsible for a fall which resulted in severe contusions of the legs. She became chilled and the next morning signs of pneumonia developed. One week later she died without further treatment. (We were notified too late to institute proper treatment).

From this case it appears that while a glycerol extract may suffice to maintain a fairly severe case, under most favorable circumstances, it will not compensate for strenuous activity or injury.

EARLY ADDISON'S DISEASE

The previous studies indicated that patients with less severe symptoms of Addison's disease might respond favorably to glycerol extract. Two such patients have been selected (Cases 4 and 5). All have shown the cardinal signs

and symptoms of the disease (asthenia, low blood pressure, gastro-intestinal irritability, abnormal pigmentation). The history and treatment of these patients are described.

Case 4. Patient O.G., a white male teacher, 52 years of age, was examined on May 15, 1935. Since 1930 this patient had noticed loss of weight and strength, easy fatigability and dizziness on standing. These symptoms were associated with low blood pressure and pigmentation of the gums. The patient noticed an increased desire for salt.

The physical examination gave negative findings except for the low blood pressure (88 mm. Hg systolic and 58 mm. diastolic) and pigmentation of the gums. The laboratory findings were essentially negative. The hemoglobin was 93 per cent.

Glycerol extract treatment was begun on May 22, 1935. The patient received 2 cc. 3 times daily. Eight days after extract was started the dizziness disappeared and there was an improved sense of well-being, increased physical activity and improved appetite. The blood pressure increased to 118 mm. Hg systolic and 80 mm. diastolic. There was a gradual disappearance of the pigmentation of the gums. Subsequently the extract was reduced to 2 cc. daily. During a period of 3 weeks while taking the reduced dosage the patient's previous symptoms slowly reappeared. The optimum dosage of the glycerol extract was found to be 2 cc. 3 times daily for this patient. Sodium chloride alone could not be substituted successfully. This patient has now been under treatment for 20 months and at the present time is able to carry on regular duties.

Case 5. Patient E.K., a white female, 42 years of age, single, was first examined on March 25, 1935. In 1933 the patient first noted that she was "turning dark." At this time she complained of muscular weakness, loss of weight and increased gastro-intestinal irritability. In 1934 a tentative diagnosis of Addison's disease associated with pulmonary tuberculosis was made. During the following year she was treated with injections of adrenal cortical extract with considerable benefit. For several months previous to treatment a condition of amenorrhea had existed. Shortly after injections of cortin were begun the normal menstrual cycle returned.

The patient was a tall, thin woman, weighing 41.8 kg. The pulse rate was 80 per minute. The blood pressure was 110 mm. Hg systolic and 80 mm. diastolic. Her skin was deeply pigmented especially about the buttocks, the upper and inner aspects of the thighs and the perineal region. There was no pigmentation of the mucous membranes. Examination of the lungs revealed persistent fine râles over the right apices with impaired resonance over the right apex posteriorly suggesting healed tuberculosis which was confirmed by an x-ray examination. The laboratory findings were essentially negative except that the CO_2 combining power of the blood was 42 vol. per cent.

Administration of 2 cc. of the glycerol extract twice daily resulted in marked improvement. An attempt to substitute sodium chloride for glycerol extract was unsuccessful due to gastric irritation. Gelatin capsules containing a mixture of sodium chloride and sodium bicarbonate were then tried. Although these were tolerated the patient lost weight and strength during 12 days of such treatment. This patient has now been treated successfully with glycerol extract for 21 months. On June 15, 1936, the patient weighed 49.0 kg. Her blood pressure was 170 mm. Hg systolic and 90 mm. diastolic with a pulse rate of 80 per minute. Subsequently this patient was ad-

mitted to the University Hospital where electrolyte balance studies demonstrated a marked retention of sodium and chloride during cortin treatment. The incompatibility of the symptoms of Addison's disease associated with a blood pressure of 110 mm. Hg systolic and 80 mm. diastolic may now be reconciled with the fact that the patient may have had a pre-existing vascular hypertension (Rowntree and Snell have noted such cases, 4).

During the next 5 months the improvement continued as far as the signs and symptoms of Addison's disease were concerned but during this time the blood pressure continued to rise and with this increase in pressure there developed the common symptoms of occipital headache and thumping in the head correlated with the pulse. On Dec. 9, 1936, the blood pressure was found to be 175 mm. Hg systolic and 110 mm. diastolic. Bronzing had disappeared completely. The patient had made a further gain of 2 kg. in weight and aside from the symptoms attributed to hypertension, felt very well.

Patients with early Addison's disease may respond so well to therapy that under continued treatment they return to normal activity. They cannot be warned too emphatically that all stresses should be avoided. The false sense of security which is experienced may lead them to be careless, even going so far as to discontinue treatment.

In addition to those patients in whom evidence points towards a diagnosis of early Addison's disease, the authors have been impressed with a larger group in whom two or more of the classical findings of Addison's disease are unexplained by any other disorder. The symptomatic response to glycerol extract has been studied in 6 such patients. Careful control of the medication, including the substitution of a placebo made the response appear to be a real one. Furthermore, glycerol extract treatment has resulted in increased ability to perform work, increased blood pressure, increased appetite, gain in weight and decreased nervous irritability. It is impossible to establish a definite diagnosis of adrenal insufficiency in these patients. However the characteristic response to extract makes the diagnosis seem highly probable.

DISCUSSION

Adrenal insufficiency is due to an inadequate supply of cortin and possibly other unknown hormones. The primary disturbance may be corrected only by hormone replacement. Adrenal insufficiency may result in a loss of sodium and chloride from the body (5). When this occurs the administration of sodium salts compensates but does not correct the change which permits the loss. Sodium therapy alone has not been sufficient to maintain most patients in optimum condition. Moreover the ultimate effect of the continued ingestion of a large amount of sodium chloride is unknown.

The body attempts to offset the loss of sodium by retaining potassium. In some instances this retention may be sufficient to cause symptoms (6). A diet of low potassium content may aid in correcting the disturbed electrolyte pattern but again does not influence the fundamental defect.

Cortical therapy is the ideal treatment for patients with adrenal insufficiency. The present cost of aqueous cortical preparations limits their use.

Glycerol extract is less expensive and may be used successfully in those patients whose cortical requirement is small. The lower cost of the glycerol extract makes continued cortin therapy possible.

SUMMARY

The use of a glycerol extract of the adrenal cortex from which the epinephrin is removed to a large extent by crystallite is described. Such a preparation is effective in early Addison's disease but has limited application in the severe stage. The lower cost of glycerol extract makes continued therapy feasible.

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FACTORS DETERMINING AND LIMITING THE GROWTH OF TRANSPLANTED SUPRARENAL CORTICAL TISSUE

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In 1932 we reported that the total amount of cortical tissue regenerating in autoplastic transplants of the suprarenal glands in rats is fairly constant for each sex, irrespective of the number of transplanted fragments present (1); furthermore, that neither autoplastic nor homoplastic transplants will grow in the presence of both or either of the rat's own suprarenal glands, or of even a good sized fragment. This limitation of the growth of transplants has been interpreted as another manifestation of Halsted's 'Law of deficiency,' namely that an animal cannot maintain a second set of organs which produce an internal secretion when in possession of its own set, and that transplantation of such organs is not successful unless there is a considerable degree of deficiency of the functional tissue in question (2). According to this view, the 'deficiency' has been taken to be the factor which determines and limits growth of transplants. We felt the need of a more exact statement of the factors involved and in our previous paper referred to them merely as a physiological or functional limitation, since we lacked data for a further explanation.

It has been established and reported in papers too numerous to quote here that the anterior lobe of the hypophysis produces a specific adrenotropic hormone, necessary for maintenance of these glands. This substance will cause hypertrophy of the suprarenal cortex when given in excess (3, 4) and apparently mediates hypertrophy of the suprarenals in certain conditions which are accompanied by such hypertrophy (5). We have investigated, therefore, the role of the hypophysis in suprarenal transplantation.

METHODS

An inbred strain of albino rats was used. Hypophysectomy was done between 3 and 6 months of age, when the average weight of males was 275 gm. and that of females was 150 gm. Completeness of hypophysectomy was judged by absence of estrus in females (vaginal smear method), testicular atrophy in males, flattening of the growth curve, and other symptoms, and established at autopsy by examination of the site. Suprarenal transplantation was done by removing both glands, bisecting them, and placing two halves in the spinotrapezius muscle at the level of the 8th or 9th rib and the other two in the retroperitoneal fat beneath the transversus abdominis just lateral to the crest of the ileum. Intramuscular autotransplantation of the suprarenal cortex

has been used successfully in this laboratory for the past 9 years, the first report appearing in 1928 (6). To check the viability of the grafts as transplants or their potency either as transplants or implants the weight curves of the animals were followed. Histological preparations of the transplants were made after autopsy whenever possible.

RESULTS

Autoplastic transplants in hypophysectomized rats. Autoplastic transplantation of both suprarenals was made in 5 rats (3 males, 2 females) approximately 3 months after complete hypophysectomy, when symptoms of hypophyseal deficiency were pronounced. All died, with symptoms of suprarenal insufficiency, from 3 to 6 days after operation. At autopsy the transplanted suprarenals were found to be degenerating. A blank operation in 3 other hypophysectomized rats, as a control for the effects of ether anesthesia and trauma in these debilitated animals, showed that removal of the suprarenals alone was responsible for the death of the transplanted rats. These controls survived over 70 days following the blank operation.

Failure of growth of the transplants may have been owing to one of three factors; lack of adrenotropic hormone, insufficiency of cortical hormone in the atrophic suprarenals (acting as implants) to carry the animal over the period until the grafts became established, or inability per se of the atrophic suprarenal tissue to grow as a transplant.

Homoplastic transplants. In order to test these factors it became necessary to resort to homotransplantation. We had previously shown that intramuscular homotransplantation of cortical tissue is successful in litter mates (100 per cent in 4 male rats) and furthermore that homoplastic transplants will grow in the presence of autoplastic transplants made at the same time (40 per cent in 10 male litter mates) (1). Martin (7) had also reported 3 successful homoplastic intra-ovarial transplants. Nilson and Ingle (8) obtained successful intra-ovarial homotransplants in sisters, but did not have success in cross-strain homotransplants.

To see if homotransplantation would be successful in our strain when the cortical tissue was contributed by non-siblings we exchanged the suprarenal glands respectively between 5 pairs of females, 3 pairs of males, and 4 pairs of a male and female each (24 rats). The members of each pair were from different parents. Successful homotransplants were obtained in 8 of the 10 females receiving tissue from another female, in 2 of the 4 females receiving tissue from a male, and in only 2 of the males. One of these received tissue from another male and the other from a female. The sex of the donor apparently made little difference, but that of the non-sibling recipient was significant since over 71 per cent of the females regenerated homotransplants whereas only 20 per cent of the males did so. Females, therefore, should be used in homotransplantation experiments when a large percentage of 'takes' is desired.

Homoplastic transplants in hypophysectomized rats. In a group of 6 male

and 9 female rats, 6 to 7 weeks after hypophysectomy, the suprarenal glands from healthy stock animals were transplanted without removing the recipient's own glands. Three males and one female died 4, 12, 12 and 20 days after operation, respectively, from hypophyseal deficiency. In these the homotransplants had degenerated. In the 11 survivors, biopsy 24 or 26 days after transplantation showed the homotransplants to be degenerated. Four females showed no significant change in weight and the other survivors, including the males, showed a slight further loss. This may be additional evidence that the homotransplants were not successful, and also that they afforded the animal no detectable benefit as implants.

This total failure of homotransplantation may have been because of one of two factors; lack of adrenotropic hormone, or inhibition of growth of the homotransplants by the presence of the recipients own glands, even though they were atrophic. The early death of over 90 per cent of hypophysectomized rats after suprarenalectomy, as described in this paper, shows that suprarenal insufficiency superimposed upon hypophyseal deficiency is rapidly fatal, and that the atrophic suprarenals of completely hypophysectomized rats are not entirely devoid of the life sustaining cortical function.

Homoplastic transplants in hypophysectomized, suprarenalectomized rats. The 11 survivors from the previous group (8 females and 3 males) and 2 other males which had been hypophysectomized for 5 months, were suprarenalectomized and transplanted with glands from healthy, young stock. All but 1 male died of suprarenal insufficiency, 11 in from 3 to 10 days and 1 female on the 21st day. The male survivor was killed on the 35th day. In all these the homotransplants had degenerated.

It might be argued that the short survival, due to superimposition of suprarenal insufficiency upon hypophyseal deficiency, did not allow enough time for the homotransplants to become established. However, from the survival of 3 animals for 10, 21 and 35 days without regeneration of homotransplants, and from comparison of the gross and histological appearance of autoplastic transplants in normal rats 4 to 6 days after operation with that of the degenerated homotransplants in the hypophysectomized series, we were led to believe that lack of adrenotropic hormone was the real reason for failure of the grafts.

Homoplastic transplants from hypophysectomized donors. In the previous series the normal donors received the atrophic suprarenal glands from the hypophysectomized rats as homotransplants. Nine normal females and 4 males were thus suprarenalectomized and transplanted. After 6 weeks there were 4 survivors in good health, these having gained weight at the normal rate. After they were killed one was found to have no functional grafts, but a small accessory was discovered in the left suprarenal site. The others had 2 or 3 small but vigorous homotransplants and no accessory cortical tissue. Histological examination showed these grafts to be organized cortical growths well laden with fine fatty granules (Ciaacio's method) and receiving an established blood supply.

The atrophic suprarenal glands of hypophysectomized rats are therefore capable of growth as homotransplants, in spite of their condition, when placed in animals having intact anterior pituitaries.

Hypophysectomy after autoplastic transplantation. In a series of rats used previously in experiments for another purpose, we had attempted to remove the posterior lobe of the hypophysis alone after they had been suprarenalectomized and had autoplastic cortical transplants well established. In those in which the anterior lobe or fragments of it had been left, the cortical transplants were later found to be growing normally. In a few in which hypophysectomy was complete the transplants showed signs of degeneration. While this observation was to be expected, it does add confirmatory evidence to the view that the adrenotropic hormone is necessary for the maintenance of cortical tissue, whether in the normal gland or transplanted. The partially hypophysectomized rats offer a control for the non-effect of an intracranial operation per se on transplanted tissue.

DISCUSSION

The results show that autoplastic cortical transplants are unsuccessful in hypophysectomized rats, although the atrophic suprarenals are capable of growth as homoplastic transplants in non-hypophysectomized rats. Homoplastic transplants of normal cortical tissue are also unsuccessful in hypophysectomized rats, either in the presence or in the absence of the recipients' atrophic suprarenals. These facts, together with other considerations discussed above, lead us to the conclusion that the available adrenotropic influence from the anterior lobe of the hypophysis is the factor which determines the growth of transplanted cortical tissue, rather than a 'need' or a suprarenal deficiency. In fact, there is evidence that cortical tissue can regenerate in the absence of a deficiency. Ingle (9) obtained regeneration of a mass of tissue about equal to the size of a normal gland from the thin layer of cells remaining after enucleation while the rats were receiving enough cortin to maintain weight and growth. We suggest, therefore, that the 'law of deficiency' as heretofore stated does not exactly apply to suprarenal transplants, but that the reason for failure of growth in the presence of already functioning self-tissue is that the available adrenotropic hormone is being used up in the maintenance of that tissue. After suprarenalectomy this hormone may be diverted to stimulate the growth of transplanted tissue. When there is a choice between already established cortical tissue, as in a single suprarenal or a good sized remaining fragment, and newly introduced transplanted tissue, the *established* tissue has the preference and the available hormone is used to induce its hypertrophy.

Limitation of the growth of cortical tissue, either in transplants or operative rests, may be explained as follows. The available adrenotropic hormone stimulates regeneration or hypertrophy, but as the tissue grows larger it begins to require the hormone for maintenance. Finally a point is reached when the available hormone is being used up for maintenance; growth then ceases. Only when it is available in excess can the hypertrophy exceed this

limit, as in conditions where the hypophysis is stimulated by various exogenous or endogenous factors (see Selye and Collip, 5). The facts that in rats the suprarenal cortex of the female is larger than that of the male, that female rats regenerate more cortical tissue in transplants or 'accessories' than do males (1), and that non-sibling homotransplants are more successful in females, suggest a greater amount or greater availability of adrenotropic hormone in females. A smaller requirement for maintenance in females would lead to the same phenomena.

It is possible that the growth of other tissues whose maintenance is determined by a specific chemical substance, such as the thyroid and the thyrotropic hormone, is similarly determined and limited by a balance between requirements for growth and requirements for maintenance acting on the available substance. This may eventually be extended to all the internally secreting organs, or even to other growth phenomena. Allen and Doisy (10) suggested that the growth and function of the ovarian follicles is restrained until puberty by the demands of rapid body growth upon available nutriment, necessary hormones, etc., and that as the growth curve flattens out this material is diverted into reproductive channels.

If adrenotropic hormone is necessary for growth of cortical tissue the possibility of tissue cultures might be questioned unless the cells possess a certain inherent power to grow in the absence of the hormone, or unless the governing factors in cultures are entirely different from those in the body. Lewis and Geiling (11) made tissue cultures of the adrenal glands from chick embryos but did not mention the presence of cortical cells, since they were interested in the epinephrine content of the medullary cells present. Lux, Higgins and Mann (12) made tissue cultures of the suprarenal glands of newborn guinea pigs and rabbits in the plasma and serum of rabbits. Epithelial tissue grew from the fragments, but they were . . . "unable to say whether any given growth from a fragment" was . . . "of cortical or of medullary origin." Apparently the question is still open.

If transplantation of cortical tissue proves to be of value in selected cases of Addison's disease, as Goldzieher and Barishaw (13) seem to have shown, knowledge of the factors determining and limiting its growth should aid in deciding upon the advisability of the operation and predicting the results. It should be noted that these authors obtained their success in a patient having *complete absence of adrenal cortical tissue* and recommended stimulation of such grafts by injection of the pituitary adrenotropic principle.

SUMMARY

In rats neither autoplastic nor homoplastic transplants of suprarenal cortical tissue will grow in animals lacking the anterior lobe of the hypophysis, although both types of graft are possible if the pituitary is present. The atrophic suprarenals of hypophysectomized rats are nevertheless capable of growth as homotransplants in suprarenalectomized rats having intact pituitaries. It is concluded that the adrenotropic hormone of the pituitary is necessary for such growth.

We suggest that the chief factor determining and limiting the growth of transplanted suprarenal cortical tissue is the available adrenotropic hormone of the host.

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EVIDENCE OF THE PROTECTIVE INFLUENCE OF ADRENAL HORMONES AGAINST TUBERCULOSIS IN GUINEA PIGS[1]

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Steinach (1) has reported that rats, a species highly insusceptible to tuberculosis, were rendered susceptible, by adrenalectomy, to bovine tubercle bacilli. We have administered adrenal products to tuberculous patients who suffered greatly with malaise and lack of vigor—some with toxemia, others without, and noted an improvement in their vigor, a lessening of their fatigue and an apparent beneficial effect on the tuberculous process. These latter effects induced us to carry out a series of experiments on guinea pigs to test whether adrenal products would exert any effect upon tuberculosis either in the susceptibility to infection or in the character of the lesion. Whole adrenal gland, eschatin, and a material made according to the method of Swingle and Pfiffner (2) were employed.

METHODS

The extracts made in our laboratory by the Swingle and Pfiffner technic represent 37 gm. of whole adrenal gland to each cc. of finished product. Their potency was proven on totally adrenalectomized cats. Guinea pigs were used for all experiments. Each was housed in an individual cage placed in a shed to protect them from the rain and direct sun. The sheds were open so that each guinea pig was exposed to the normal variations of temperature and humidity.

Every inoculated guinea pig received an intraperitoneal injection of 100 ± 10 tubercle bacilli, freshly obtained from human sputa, the number being determined by the method of Pottenger (3). The animals were weighed weekly. After death they were autopsied by number, their identity being unknown to the pathologist. The extent of the tuberculous process in the animals was judged clinically by the degree of enlargement of the lumbo-sacral lymph nodes as palpated through the abdominal wall; the general condition of the animal; and, in the male cavy, by the appearance of testicular involvement.

At autopsy, the nutrition was estimated from the amount of body fat. It was recorded as follows: 0, total absence of fat; 1+, slight omental and retroperitoneal fat; 2+, moderate fat in abdominal structures; 3+, sufficient fat to interfere with examination.

The extent of the lesion at autopsy was recorded according to the following scale; 0, no infection; 1+, less than 10 nodules in the abdomen; 2+, moderate abdominal involvement with not more than minor thoracic involvement;

[1] Read before the Twentieth Annual Meeting of The Association for the Study of Internal Secretions, Kansas City, Missouri, May 11, 1936.

3+, moderate thoracic involvement with more or less extensive abdominal involvement; 4+, extensive involvement of both abdominal and thoracic cavities.

The criterion of infection at autopsy was the recovery of the tubercle bacillus from the tissues. Where the examination revealed no obvious evidence of tuberculous infection, selected bits of tissue were subjected to the dilution-flotation technique. A weighed amount of tissue was ground until dry; rubbed with 8 to 10 volumes of 0.5 per cent NaOH; and after being digested for one hour at 55°C. the original material was diluted with distilled water, 300 times for fibrous tissues and 500 times for vascular tissues; then 3 to 5 cc. of xyol was added. The material was agitated in a mechanical shaker for 30 minutes. A layer of soap arose to the surface when the material was allowed to stand. A slide was prepared from this surface material and searched for tubercle bacilli for 20 minutes before the specimen was declared negative.

RESULTS

Series I. On February 14, 1933, 36 guinea pigs were divided into 7 groups. Six animals served as controls to determine longevity and variations in weight. Five were inoculated but not treated. Five received orally 1 gm. of whole raw adrenal gland twice a week. Five were uninoculated and received 0.2 U. of insulin daily.[2] Five were inoculated and received 0.2 U. of insulin daily, until death. Five were inoculated and treated with 0.2 U. of insulin and 1/150 cc. of 'batch H' cortical hormone daily until death. Five were inoculated and received 1/150 cc. of batch H cortical hormone daily until death.

Five of the untreated control animals are now dead, after an average survival period of 485 days. One animal is still alive at the end of 1440 days. The 5 dead animals showed an average spleen weight of 0.84 gm., averaged 3+ fat, and showed no evidence of any tuberculous infection either grossly or upon microscopic examination.

The 5 inoculated control animals all became infected. They showed an average life of 130 days; an average of 1.2+ fat and the spleen weight averaged 14.00 gm. They had predominantly caseous lesions and averaged 3.2+ infection.

Of the 5 that were inoculated and received 1 gm. of raw adrenal gland orally twice weekly all became infected and lived an average of 178 days. Their spleens averaged 2.1 gm.; they showed an average of 1.6+ fat, had predominantly fibrocaseous lesions, and showed 3.2+ infection.

Of the 5 animals receiving 0.2 U. of insulin daily, 2 are still alive at the end of 1440 days; the 3 which came to autopsy had an average of 0.8 gm. spleen weight, and none showed any evidence of tuberculous infection.

The 5 inoculated animals receiving insulin all became infected and survived an average of 279 days. Autopsy showed: 1.6+ fat and average spleen weight of 6.7 gm.; lesions predominantly fibrous and infection 3.4+.

The 5 inoculated animals receiving both 0.2 U. of insulin and 1/150 cc. of batch H cortical hormone all became infected and lived an average of 146

[2] Insulin donated by Sharpe and Dohme Company.

days; had spleen weights averaging 2.6 gm.; averaged 3.0+ fat; had predominantly the glandular type of lesion; and averaged 3.0+ infection.

The five guinea pigs inoculated and receiving 1/150 cc. batch H cortical hormone survived on the average 183 days. Three showed an average spleen weight of 4.4 gm.; had predominantly fibrous lesions; had 1.8+ infection, and had tuberculous disease. Two of this group, however, had spleen weights averaging 0.8 gm. and showed no evidence of tuberculous infection.

Series II. The second series of 40 guinea pigs was started September 18, 1933. The treatment was 1/100 cc. instead of 1/150 cc. of the cortical adrenal extract of the same batch as that used in the original experiment, for a period of 250 days. Ten were kept as normal controls, 10 were inoculated but received no treatment, 10 received cortical adrenal extract daily but were not inoculated and 10 were inoculated and received cortical extract daily. An extreme heat wave resulted in the death of 1 animal in each of the first 3 groups and 2 in the fourth group.

Four of the normal controls have died. They lived an average of 584 days and at autopsy showed spleen weights of 0.5 gm., and an average of 2.3+ fat. They showed no evidence of tuberculous infection. Five of the animals are still alive at the end of 1230 days.

Of the group of inoculated, untreated animals, 9 finished the experiment, all became infected. They lived an average of 389 days; showed an average of 2.1 gm. spleen weight; 1.4+ fat; and 3.1+ infection.

Nine uninoculated animals receiving the cortical extract for the period of 250 days are to be counted in the experiment. Of these, 6 lived an average of 583 days. At death they showed an average of 0.7 gm. spleen weight; 2.5+ fat; and no evidence of tuberculous infection was found. Three of these animals are still alive at the end of 1230 days.

Of the group inoculated and receiving cortical adrenal extract, 8 survived. Of these, 6 animals were infected. They lived an average of 318 days. At autopsy they showed an average of 4.4 gm. spleen weight; 1.8+ fat, and 2.3+ lesion. One animal showed no evidence of tuberculous disease on clinical or gross pathological examination, but on pooling the spleen and the glands of the abdomen, one tubercle bacillus was found in a 20 minute search, after these tissues had been subjected to the dilution-flotation technique. Two of the group lived an average of 786 days; showed an average of 1.1 gm. spleen weight; 2.5+ fat; and showed no evidence of tuberculosis clinically, at autopsy, or on examining the tissues by the dilution-flotation technique.

Series III. This series was started June 6, 1934. The animals were treated with 1/100 cc. cortin (eschatin).[3] The series consisted of 40 animals; 10 normal controls; 10 inoculated and untreated; 10 received cortin for 10 days before inoculation; 10 received cortin for 10 days after inoculation; and 10 received the cortin for 10 days before and 10 days after inoculation. Many of the animals were killed by an intense heat spell before expiration of the time intended for the termination of the experiment but none died before sufficient time had elapsed for them to be infected, if they were to become so.

[3] Eschatin donated by Parke, Davis and Company.

None of the 10 controls developed tuberculosis; the spleens average 0.8 gm. The 10 inoculated but not treated all became infected. They showed spleen weight of 1.1 gm., and averaged 2+ infection. Of the 10 animals receiving cortin for 10 days before inoculation, 8 showed infection. These had 0.92 gm. average spleen weight and 1.5+ infection. Of the 10 animals receiving cortin 10 days before and 10 days after inoculation, 6 were found infected at autopsy. They showed an average spleen weight of 0.9 gm., and had 2.0+ infection. Of the 10 animals receiving cortin for 10 days after inoculation, 8 showed infection. These had an average spleen weight of 0.8 gm. and 1.9+ lesion.

Combining the 3 series it was found that 20 uninoculated, untreated animals had spleens averaging 0.79 gm. in weight, and none showed evidence of infection. Of 23 animals which received no treatment, inoculated with 100 ± 10 tubercle bacilli obtained from fresh human sputa, the spleens averaged 3.97 gm. in weight; and all of the 23 were infected. Of 6 uninoculated animals which received cortin, the spleens averaged 0.73 gm. in weight and none of the animals showed evidence of infection.

On the other hand, of 43 guinea pigs inoculated with 100 ± 10 tubercle bacilli obtained freshly from human sputa, but which received cortin for periods of from 10 days to the life-span of the animal, the spleen weights at death averaged 1.69 gm. Of these 43, 12 showed no evidence of tuberculous infection and one of the remaining 31 animals showed neither clinical nor gross pathological evidence of infection, although upon subjecting the tissues to the dilution-flotation technique, one tubercle bacillus was found after a 20 minute search.

SUMMARY

Experiments are detailed which show that extracts made from the adrenal gland containing cortin exert an inhibiting influence on tuberculous infection in guinea pigs.

Three series of experiments are presented in which longevity and the extent and nature of the disease found at autopsy in animals treated with cortical extract are compared with untreated controls and animals treated with other substances.

Of 24 untreated animals inoculated with 100 ± 10 tubercle bacilli obtained from fresh human sputa and 5 inoculated but treated with insulin all became infected.

Twelve of 43 inoculated animals (28 per cent) which received cortical extract for periods varying from 10 days to the entire life of the animals showed at autopsy no evidence of tuberculous infection. It was further found that infection, when present, differed from that in untreated animals in that it was milder and less extensive.

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THE SPECIFICITY OF PROGESTERONE IN INDUCING SEXUAL RECEPTIVITY IN THE OVARIECTOMIZED GUINEA PIG[1]

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The induction of sexual receptivity in the ovariectomized guinea pig by progesterone following proper conditioning by theelin has been recently described by Dempsey et al. (1). These workers regard the elicitation of the copulatory reflex as the essential criterion of estrus, and have adequately described the phenomenon in earlier studies (2). The data here presented indicate the distinct specificity of progesterone as compared to several closely related substances in producing the copulatory reflex in the guinea pig. Seven compounds having the cyclopentenophenanthrene nucleus characteristic of the sex hormones were assayed and found to be entirely without effect. In addition, we have investigated the effectiveness of progesterone at varying dosage levels and have confirmed the observations of Dempsey et al. (1).

MATERIALS AND METHODS

The guinea pigs used in these studies were young adult virgin females which had been ovariectomized at least 1 week preceding their experimental use. One week also intervened between 2 treatments of the same animal.

All animals were first given 1 γ of crystalline theelin in 0.25 cc. of corn oil twice daily for 2 days. On the following morning the substance to be assayed was administered in 0.25 cc. of corn oil. The animals were then examined at 1 to 2-hour intervals for a period of 10 hours from the time of this injection. Along with each group of animals given the compounds of unknown effectiveness, a small number of control animals given either synthetic crystalline progesterone or active corpus luteum extracts were tested for the copulatory reflex.

The substances to be tested were administered first in the same absolute dose known to be adequate for uniformly positive results with progesterone, i.e., 0.2 I.U. (0.2 mg.) (1). When this amount was found to be ineffective it was increased 4-fold to determine whether the compound in question is effective in larger quantities.

Pregnandione (m.p. 121°) and progesterone were synthesized from pregnandiol according to the method of Butenandt and Schmidt (3). The progesterone was used in the form of a mixture of alpha and beta modifications (m.p. 116-123°). Androstendione, (m.p. 167°) was made according to Butenandt and Kudszus (4), and dehydroandrosterone (m.p. 146°) as directed by Wallis and Fernholz (5). Luteosterone A (allo-pregnanol-3-1-20,

[1] Aided in part by a grant from the Wisconsin Alumni Research Foundation.
[2] Chappel Bros. Fellow in Endocrinology.

m.p. 190°) was kindly supplied by Dr. Willard Allen. We are indebted to Dr. E. E. Schwenk of the Schering Corporation for testosterone and a part of the progesterone, and to the Upjohn Company for the crystalline oestrone (m.p. 251°), oestriol (m.p. 276° and pregnandiol (m.p. 251°). The estrin-free corpus luteum extracts were prepared from sow's ovaries by the method of Allen and Meyer (6).

RESULTS AND DISCUSSION

The data obtained with the 7 steroids investigated are presented in table 1. These substances were chosen for their relatively close chemical relationship to progesterone. In view of the uniformly positive responses obtainable with

TABLE 1. RESULTS WITH EFFECTIVE DOSES OF PROGESTERONE AND CORPUS LUTEUM EXTRACTS AND COMPARABLE AMOUNTS OF RELATED COMPOUNDS.

Compound or Extract	Dose	No. of Animals	No. Positive	No. Negative
Pregnandione	0.2 mg.	3	0	3
Pregnandione	0.8 mg.	4	0	4
Pregnandiol	0.2 mg.	3	0	3
Pregnandiol	0.8 mg.	3	0	3
Luteosterone A	0.2 mg.	4	0	4
Luteosterone A	0.8 mg.	4	0	4
Oestriol	0.2 mg.	8	0	8
Oestriol	0.8 mg.	8	0	8
Progesterone (crystalline)	0.2 mg.	5	5	0
Progesterone (crystalline)	0.1 mg.	6	6	0
Sow corpus luteum extract P-25	0.2 RB.U.	8	8	0
Sow corpus luteum extract P-25	0.1 RB.U.	6	5	1
Sow corpus luteum extract P-27	0.2 RB.U.	10	10	0
Sow corpus luteum extract P-27	0.1 RB.U.	9	9	0
Androstendione	0.2 mg.	4	0	4
Androstendione	0.8 mg.	4	0	4
Dehydroandrosterone	0.2 mg.	4	0	4
Dehydroandrosterone	0.8 mg.	3	0	3
Testosterone	0.2 mg.	4	0	4
Testosterone	0.8 mg.	4	0	4

progesterone, a small group of 3 or 4 test animals giving uniformly negative results was deemed adequate to demonstrate the ineffectiveness of a given compound at each dose level.

In 60 trials with the compounds chemically related to progesterone, not one positive response was obtained, whereas in 44 trials with from 0.1 to 0.2 I.U. of progesterone or equivalent amounts of corpus luteum extract positive results were obtained in all but 1 case. We feel that such data demonstrate the specificity of the copulatory response for progesterone under the conditions of dosage and time here employed. In pointing out the specificity of progesterone under these experimental conditions, one should not disregard the fact that in large doses theelin alone can produce the copulatory reflex under other conditions described by Dempsey et al. (1).

The latent period in the progesterone-treated animals varied from 3½ hours to 7¼ hours, corresponding well with the latent periods of 3 to 9 hours reported by Dempsey et al. (1). Our combined data establish the adequacy of a 10-hour test period for the detection of positive responses.

We have summarized in table 2 the results obtained with doses of progesterone of 0.05 I.U. and 0.025 I.U. and equivalent amounts of 2 corpus luteum extracts. It is apparent that the use of the copulatory reflex as an end point allows one to detect as little as 0.05 I.U. of progesterone, although the results vary somewhat from one series to the next. Half of this amount gives only an occasional response, presumably indicating variation in the responsiveness of the animals.

In view of the sensitivity and specificity of this method we propose to employ it to detect progesterone in various tissues and body fluids. The copulatory reflex offers a specific biological test enabling one to demonstrate approxi-

TABLE 2. RESULTS WITH LOW DOSES OF PROGESTERONE AND CORPUS LUTEUM EXTRACTS.

Substance	Dose	Series No.	No. of Animals	No. positive	No. negative
Progesterone (crystalline)	0.05 mg.	1	6	5	1
	0.05 mg.	2	6	5	1
	0.05 mg.	3	6	4	2
	0.025 mg.	1	16	3	13
	0.025 mg.	2	14	3	11
	0.025 mg.	3	6	3	3
Corpus luteum extract, P-25	0.05 RB.U.	1	6	4	2
	0.05 RB.U.	2	6	4	2
	0.025 RB.U.	1	6	0	6
	0.025 RB.U.	2	6	2	4
Corpus luteum extract, P-27	0.05 RB.U.	1	6	3	3
	0.05 RB.U.	2	6	3	3
	0.025 RB.U.	1	8	0	8
	0.025 RB.U.	2	8	1	7
	0.025 RB.U.	3	6	2	4

mately one tenth of the amount of progesterone required for bioassay by the other methods available (7) (8). This should prove advantageous in investigating the progesterone content of biological materials.

SUMMARY

Seven compounds closely related to progesterone (pregnandione, pregnandiol, luteosterone A, estriol, androstenedione, dehydroandrosterone, and testosterone) proved unable to produce the copulatory reflex in the theelin conditioned, ovariectomized guinea pig.

Synthetic crystalline progesterone and estrin-free, sow corpus luteum extracts in adequate dosage proved uniformly effective. Assays of lower doses of progesterone using sexual receptivity in the guinea pig as an end point can detect as little as 0.05 I.U. of progesterone. The method is consequently offered as a sensitive and specific assay for progesterone.

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THE EFFECT OF ARTIFICIALLY RAISED METABOLIC RATE ON THE ELECTRO-ENCEPHALOGRAM OF SCHIZOPHRENIC PATIENTS[1]

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From a study of the '10 per second' (alpha) rhythm from the human occipital cortex in relation to temperature variations, Hoagland (1) pointed out that the frequency of the alpha waves under standard conditions is apparently proportional to the rate of respiration of the brain cells. The direct relationship of the lowering of blood sugar (2) to the alpha frequency is in accord with this view. Santenoise (3) has produced evidence which indicates that the thyroid apparatus is involved in cerebral activity. The present report is concerned with the investigation of this problem by the study of the effects on alpha frequency of elevation of the basal metabolic rate by massive doses of thyroxin. This paper is also part of a larger study, now in progress at the Worcester State Hospital, on the physiological and psychiatric changes in deteriorated schizophrenics by hyperthyroidization and hyperthyroxinization.

PROCEDURE

Four schizophrenic patients were used as subjects. Electro-encephalograms and basal metabolic rates (B.M.R.) were recorded simultaneously.[2] Data were first obtained for each patient 2 or 3 times per week over a period of about 3 weeks. The patients were then given daily intravenous injections of thyroxin.[3] The dosage for the first week was 2.5 mg. per day and was increased to 3.75 mg. for the remaining period of medication (approximately 3 weeks). The experiment was continued for about 4 weeks after medication was stopped. Some 3 tests per week were made on each patient over the experimental period of about 2½ months, roughly 30 tests per patient.

The recording electrodes consisted of flattened pellets of lead solder, 2 to 3 mm. in diameter, attached to fine enamel-insulated copper wire. The active lead was placed about 2 cm. above the inion and fixed to the scalp by collodion. An electrode was placed over the mastoid process on each side and connected in parallel to form one indifferent lead. Electrical contact for both leads was made with electrode paste. The electrodes were held firmly in place over the mastoid processes by strips of surgical tape. The electro-encephalograms were registered on paper tape by means of an amplifier and undulator system.

[1] This work has been aided in part by a grant from the Rockefeller Foundation.

[2] All the B.M.R. determinations were made by Miss Anna Walsh, to whom we are indebted for kind and painstaking assistance.

[3] The thyroxin was generously supplied by E. R. Squibb and Sons, New Brunswick, New Jersey.

The patients, who rested in bed in the basal state in a quiet, partially darkened room, were instructed to lie quietly with eyes closed while the records were being taken. A constant amplification of 10 microvolts per 1.5 mm. deflection was used throughout the experiments.

RESULTS

It was found that during the period before the administration of thyroxin both the alpha frequency and the B.M.R. varied in a random manner over a narrow range. Within 2 days after the first injection of thyroxin the alpha frequency and B.M.R. increased rapidly in 3 of the patients. The rise was continual and rapid for about a week, after which its level remained relatively

Patient	Conditions of Medication	Mean Alpha Frequency	Range of Alpha Frequency	Mean B.M.R.	Range of B.M.R.	Increase in mean Alpha Frequency	Increase in Mean B.M.R.
						%	%
J.H.	No thyroxin	10.3	10.2–10.4	88	84– 90	17.2	47.7
	Thyroxin	12.1	11.6–12.6	130	106–154		
P.B.	No thyroxin	10.3	10.0–10.5	95	94– 95	9.8	36.6
	Thyroxin	11.3	10.9–11.8	131	93–173		
V.C.	No thyroxin	9.6	9.3– 9.9	83	79– 90	4.9	26.5
	Thyroxin	10.1	9.9–10.2	105	97–113		
L.A.	No thyroxin	12.2	12.1–12.5	83	79– 90	7.6	26.5
	Thyroxin (1st week)	13.2	13.0–13.3	105	96–114		
	(remainder of medication period).	12.2	12.0–12.6	115	110–119	0.0*	38.5*

* Obtained by subtracting the 'thyroxin' values from the 'remainder of medication period' values.

constant throughout the entire period of medication. The maintenance of a constant high level appears noteworthy, since the daily thyroxin dosage was increased to 3.75 mg. after the first week. The fourth patient (L.A.) exhibited an initial rise in both B.M.R. and alpha frequency, but the latter dropped to the pre-medication level after the first week despite the daily administration of thyroxin in the same dosages as those for the other patients; during the remainder of the medication period the B.M.R. was maintained at a level 38.5 per cent higher than the pre-medication B.M.R.

The table presents the data obtained from the 4 patients. The data on alpha frequency are mean values obtained by averaging the means of some 40 to 50 seconds of each daily record under pre-medication and medication conditions. These data may be compared with the means of the B.M.R. obtained under the same conditions. The range of values obtained for each of these means is also presented. The percentage increases in alpha frequency as well as in B.M.R.

are shown; these increases are significant, since there is no overlap between the pre-medication and medication ranges of alpha frequencies. Evidently we are dealing with mean values of entirely different statistical populations.

From the table it may be seen that in 3 cases there is a direct relationship between alpha frequency and B.M.R. in that both variables were affected in the same direction on thyroxin administration. These data corroborate and extend the observations of Gerard (personal communication), who has observed an increase in alpha frequency in several 'normal' individuals on administration of thyroid, although in one case an increase of over 35 per cent in B.M.R. was not accompanied by any change in alpha frequency. A possible explanation of Gerard's and our 'exceptional' cases may lie in the fact that many physiological variables react biphasically on thyroxin (or thyroid) administration if their initial level is higher than the average 'normal' level; that is, instead of increasing rapidly and then reaching a relatively constant high level, there is an initial increase and then a rapid falling off to the original level. An example of this can be found in a paper by Hoskins and Jellinek (4) on the erythrocyte count during continued high thyroid administration. Apparently patient L.A., who is our 'exceptional' case, has a pre-thyroxin alpha frequency which is approximately the physiological limit for that variable, and any increase of that initial high level cannot be maintained for very long. (The range of alpha frequencies commonly observed in man is from 8 to 12 per second; L.A.'s frequency is 12.2 per second.) Jasper (personal communication) has also observed an increase of 10 to 15 per cent in alpha frequency in an individual to whom thyroid was administered.

Lindsley and Rubenstein (5) have recently reported a high correlation between alpha frequencies and total oxygen in 13 normal adults. A good correlation was also observed between these variables in one subject who received a single injection of 1.6 mg. of thyroxin. They did not find a significant inter-individual correlation between B.M.R. and alpha frequency. We do not find an inter-individual correlation between alpha frequency and B.M.R. in non-thyroxinized patients. For example, although patients V. C. and L. A. both have a B.M.R. of 83 before thyroxin administration, the alpha frequency is 9.6 per second for V. C. and 12.2 for L. A. On the other hand, when the B.M.R. in a given individual is increased by thyroxinization an increase in alpha frequency is found. Intra-individually, then, there is a direct correlation between B.M.R. and alpha frequency.

The post-medication data are not included in the table, nor dealt with here, for the following reasons. In one patient (V.C.) both the average B.M.R. and alpha frequency, for about 10 days after medication had ceased, were higher than during the period of thyroxin medication; from then on a steady decline to the level of the pre-medication values occurred. In patients P.B. and J.H. high values in B.M.R. and alpha frequency were obtained for a month after the discontinuation of thyroxin. Another fact which made an evaluation of the post-medication data impossible was the poor cooperation of patients P.B. and V.C. during this period.

Because of the possibility that increased B.M.R. might be accompanied by

an increase in body temperature sufficient to account for the observed increase in alpha frequency, rectal temperatures were obtained daily just before each experimental session. The temperature variations were found to be very small; the greatest variation (difference between the lowest and highest values) observed in the patients was 1.0°F. From the data of Hoagland (1), the change in alpha frequency which might be expected for such a change in temperature is 1.5 per cent, obviously very small in comparison with that found as a result of thyroxin administration.

DISCUSSION

Although the basal metabolic rate reflects the metabolic activity of body tissue (primarily muscle) which in bulk is far in excess of brain tissue, it is reasonable, nevertheless, to infer that an increase in B.M.R. involves an increase in the rate of brain respiration. Experiments by R. A. Cohen (6) on the respiration of the brain of thyroidized rats lends support to this statement. Cohen found an appreciable increase in the brain Q_{O_2} of rats fed with desiccated thyroid, which he attributed especially to increased carbohydrate oxidation. That the rate of carbohydrate oxidation in the brain is an important factor controlling the alpha frequency during severe hypoglycemia induced by insulin has been shown by Hoagland, Rubin, and Cameron (2). They demonstrated that the marked lowering of blood sugar by administration of insulin was associated with a decrease in alpha frequency of as much as 40 per cent. Kerr and Ghantus (7) have shown that insulin hypoglycemia lowers the carbohydrate level in mammalian brain. The diminution in alpha frequency in man lags behind the blood sugar curve, as does the restoration of the frequency after injection of glucose. This lag is roughly of the same order of magnitude as the lag between blood sugar and brain sugar in dogs and rabbits after the administration of insulin.

It would appear, then, that alpha frequency measurements during artificially induced hyperthyroidism (and during severe insulin hypoglycemia) are indirect measures of carbohydrate oxidation in brain tissue. It should not be inferred from this statement that carbohydrate need or cell respiration is the only factor involved in alteration of the alpha frequency, but, from other evidence, such as the identity of temperature characteristics for alpha frequencies with the principal ones for cell respiration in vitro, the effects of insulin and subsequent glucose administration in lowering and restoring the frequency, one may hold the view (1) that the basic frequency of the alpha waves, in the absence of extraneous variables, is proportional to the rate of respiration of the brain cells producing the alpha rhythm.[4]

SUMMARY

The effect on the alpha frequency of artificially raised basal metabolic rate (by hyperthyroxinization) has been studied in 4 schizophrenic patients.

In 3 of the patients the alpha frequency was increased 4.9 to 17.2 per cent

[4] For a review and discussion of physiological rhythms in relation to continuous chemical events see Hoagland (8).

when the B.M.R. was increased 26.5 to 47.7 per cent. The fourth patient showed an initial increase in alpha frequency accompanying an increase in B.M.R.; after about a week, however, despite a continued high B.M.R., the alpha frequency fell to the pre-thyroxinization level.

In general the raised metabolic rate was associated with a rise in alpha frequency. A constant high level for both these variables was maintained during thyroxin medication despite an increase in dosage and the continuation of thyroxin, and was maintained for a lengthy period after thyroxinization was discontinued.

The present data, in the light of other investigations, lend further weight to the hypothesis that there exists a quantitative relationship between alpha frequency and carbohydrate metabolism of the brain.

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THE INFLUENCE OF FASTING ON THYROPARATHYROIDECTOMIZED ALBINO RATS

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Since there is a wide variation in the mortality and morbidity observed in different laboratories following thyroparathyroidectomy of albino rats, it is important to know to what extent postoperative treatment during the first 24 hours affects the results. Barnes and Fogelberg (1) reported that a marked variation occurs in the mortality of a given age and necessitates the use of a large number of animals to justify conclusions. Chandler and Hughes (2) did not observe this variation in mortality provided the animals were returned to an adequate diet immediately after the operation and the temperature of the room did not exceed 80° F.

In the most common postoperative procedure animals are kept in a warm room and in a fasting condition for several hours. The importance of the influence of temperature, without reference to food intake, has been reported by several investigators (3, 4, 5). We (6) reported in 1935 a high incidence of tetany at high environmental temperature and a low incidence at low temperature in thyroparathyroidectomized albino rats subjected to 24 hours of fasting. From this work it was notable that, with the effect of food eliminated by fasting, lowering of the environmental temperature by approximately 15° F. (from a range of 80-93° F. to a range of 63-80° F.) greatly reduced the mortality although there was a high morbidity. Further lowering of the temperature, to a range of 54-70° F., also reduced the morbidity.

In this work the temperature factor was kept constant while studying the influence of fasting. Prior to thyroparathyroidectomy, the 177 albino rats used were kept on a diet of Fox Chow, ad libitum with bread, meat and cabbage once per week. They were weaned at the age of 21 days, and thyroparathyroidectomized between the ages of 22 to 33 days. Littermates of the same sex and as nearly the same weight as practical were divided into experimental groups.

Immediately following the operation, the animals were taken to a room where the temperature, thermostatically controlled, varied from 88 to 92° F. During the succeeding 24 hours, 81 of the animals received Fox Chow while 96 were subjected to fasting. During this period the animals were observed 3 times, namely about 12, 18 and 24 hours after the operation. At each observation time the animals were classified as *1)* surviving without tetany, *2)* surviving with tetany, and *3)* dead. The difficulty in determining mild tetany

made it necessary to establish an arbitrary rule, namely, if a definite rigidity existed at the time of observation in one or more limbs or developed after one half minute of exercise, the animal was considered as being in tetany. Because of difficulty in applying this arbitrary rule the mortality figures are obviously the most accurate.

In the first 12 hours (table 1) the animals which received food following the operation showed a mortality of only 10 per cent, while 9 per cent survived with tetany and 81 per cent survived without tetany. The importance of food during this period is revealed by comparing these percentages with the result obtained on littermates which did not receive food. Under this fasting condition, there was a mortality of 40 per cent while 30 per cent survived with tetany, and 30 per cent survived without tetany. In the fasting animals mortality was 30 points higher and tetany 20 points higher than the percentage of

Table 1.

Post-operative treatment	Total number of animals		First 12 hours			First 24 hours		
			Surviving without tetany	Surviving with tetany	Dead	Surviving without tetany	Surviving with tetany	Dead
Fed	81	Number	66	7	8	53	12	16
		Per cent	81	9	10	65	15	20
Fasting	96	Number	29	29	38	14	25	57
		Per cent	30	30	40	15	26	59

animals which received food ad libitum. The percentage of animals surviving without tetany in the fasting group was only 30 as compared to 81 in the group receiving food.

When the animals received food ad libitum during the course of the experiment, the percentage of deaths was as great in the second-hour period as in the first, but more tetany occurred in the first 12 hours. Under the fasting condition, the mortality in the first 12-hour period was approximately 20 points above that of the second. The percentage of animals surviving with tetany was greater in the first 12-hour period than in the second, due to death in the second period of many of the animals previously recorded as surviving with tetany. In both groups of animals no significant difference could be noted between the sexes which were nearly equally divided.

A summary of the results for the entire 24 hours (table 1) following thyroparathyroidectomy revealed that 20 per cent of the animals which received food died, 15 per cent survived with tetany, and 65 per cent survived without tetany. When the animals did not receive food 59 per cent died, 26 per cent survived with tetany, while only 15 per cent survived without tetany. The mortality for this period was 39 points higher and the tetany 11 points higher in the fasting animals. The percenage of animals which survived without tetany in the fasting group was only 15 per cent as compared to 65 per cent in the group receiving food.

Our observations may be explained in the light of the findings of Blumenstock, Tweedy and Brannon (7). They studied calcium metabolism in a thyroparathyroidectomized dog (which also had an Eck fistula). At the time of the operation the plasma calcium was 11.5 mg. per cent. During the succeeding 4 months, the plasma calcium fluctuated between 4.35 and 7.55 mg. per cent (8). At the close of that period, food was withheld for 48 hours and the plasma calcium fell from 6.67 to 3.71. Associated with the fasting, shivering and muscle tremors were observed which abated after feeding the animal 50 gm. of beef bone.

The variability in the mortality and morbidity of albino rats following thyroparathyroidectomy is commonly interpreted as due to the variability in the presence of, or the quantity of accessory parathyroid tissue. By serial sections of the neck region, Hoskins and Chandler (9) were able to find accessory parathyroid tissue in only about 11 per cent of 44 adult albino rats. Many investigators, however, are not convinced that this method is adequate to determine the presence or absence of accessory tissue in all cases. Granting that accessory parathyroid tissue exists in some cases, one must assume that such tissue is functional—an assumption that has not been proven.

Wide variations in results can be explained if due consideration is given to environmental temperature and postoperative feeding. With these two factors reasonably constant the mortality and morbidity, following thyroparathyroidectomy of different groups of rats in a given age range, will have only a small variation. Even this small variation may be explained by other experimental variables which will be discussed in a later communication.

SUMMARY

A study of the influence of fasting on 177 thyroparathyroidectomized albino rats was made with the environmental temperature maintained at 88-92° F. The return of the animal to food immediately following thyroparathyroidectomy was found to decrease the mortality and morbidity in the first 24 hours following the operation.

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CASE REPORTS

CLINICAL EXPERIENCES WITH PROTAMINE INSULIN IN ITS VARIOUS MODIFICATIONS[1]

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Protamine insulin although already extensively used in this country, is still in its experimental stage. Experiences are reported here, based on the use of this new product and its several modifications in about 100 patients with diabetes of various degrees of severity. Some of these had been under my close supervision for a number of years previous to the development of the new type of insulin and therefore presented a good group for study. Some had been treated with insulin before. Protamine insulin[2] has been used since January 1936 and several methods with the various protamine preparations as well as with crystalline insulin have been tried on the same patients for long intervals.

The most of the patients were treated in the clinic and at the office; only a small number were hospitalized. The use of insulin in ambulatory cases presents problems not encountered in hospital patients. While the observations made on these patients were not under absolutely controlled conditions, the conclusions are of practical importance since the patients have been closely followed during their daily routine of life.

At the first the most intelligent and reliable patients were chosen. Greater caution in the ambulatory case was necessary; the initial dose as well as the increase in dosage was smaller. Each ambulatory patient was given a chart and was instructed to record daily the result of the urine examination, the amount and kind of insulin he was using, and the type and time of reaction. The urine was tested at least 3 times a day and microcapillary blood-sugar determinations were carried out frequently at various hours of the day. With proper instructions the patients encountered no difficulties in the use of protamine insulin. With the introduction of the mixed protamine insulin its administration has been further simplified.

REPORT OF CASES

A few cases have been selected for detailed presentation in order to illustrate the gradual development of the present method of treatment, and of vari-

[1] Aided by a grant from the Charlton Research Fund, Tufts College Medical School.

[2] The protamine insulin used in this work was generously supplied by Eli Lilly & Co., and also later by E. R. Squibb & Sons, and Sharp & Dohme (Mulford). It consisted at first of 3 kinds: plain protamine insulin, protamine insulin with zinc or calcium unmixed, and now protamine-zinc-insulin mixed. The zinc content is now 0.08 mg. per 100 U of insulin. The crystalline insulin was received through the courtesy of Frederick Stearns & Co.

ous problems that might arise with the introduction of protamine insulin.

Only a few of the blood sugar values determined during the various periods of treatment have been recorded in the charts, to illustrate the blood sugar curves during the 24 hours. The letters R, SR, and VSR, indicate a mild, severe or very severe reaction. The unitage of the new insulin, protamine or crystalline, is given in italics to distinguish these preparations from regular insulin.

Case 1. A. C., a male, age 39, had had diabetes mellitus since 1924. He was first seen in the Out-Patient Department of the Beth Israel Hospital in 1930. He was found to be a severe diabetic with a greatly fluctuating blood sugar, and subject to violent insulin reactions. Under close supervision, and with great effort, he was finally regulated on a diet consisting of C-180, P-100, F-100, cal.-2020, with 3 doses of regular insulin daily, specially timed, namely 22 U 1 hour before breakfast, 6 U before lunch and 22 U 1 hour after supper.

In Feb. 1936 I gave him a trial with the protamine insulin. The total of his diet remained unchanged throughout the entire period reported here. It was necessary, however, to divide his diet in frequent feedings during the day to avoid insulin reactions. Chart 1 illustrates the results obtained with the new insulin as compared with the regular.

Various methods of treatment have been tried for fairly long intervals. On all the regimes he had some severe insulin reactions. He was best regulated, however, when he was given a single dose of protamine-zinc-insulin daily before breakfast.

This is an example of a severe case of diabetes mellitus which benefited from the introduction of protamine-zinc-insulin. He now requires only one dose of protamine-zinc-insulin daily instead of 3 doses of regular insulin, specially timed. See chart 1.

Case 2. H. B., a female, age 32, was first seen Sept. 12, 1936. Her diabetes was discovered 4 years previously and she was treated by several physicians on diet with insulin. Her diet was irregular and the insulin dosage was inadequate. She was not feeling well and was losing weight. On September 1 she developed epigastric distress, which lasted for 3 days. Her family physician gave her powder with relief and reduced her insulin from 20 to 15 U regular twice daily. When seen by me all her single urine specimens during the 24 hours showed +4 sugar and no acetone. Physical examination disclosed no abnormalities. On a diet of C-150, P-80, F-60, cal.-1460, she was given 30 U of regular insulin before breakfast and 10 U of regular insulin followed by 20 U protamine-zinc-insulin before supper. By mistake, however, she took for several days twice the amount of insulin ordered. Chart 2 gives the amount she was taking. When the error was discovered, the patient was allowed to continue with the large doses of insulin, since she was doing well on this program. Various methods have been tried and it was found that she could get along well on a single dose of the protamine-zinc-insulin. After a short while, during which time she only had a few mild reactions, she was regulated on 68 U of the protamine-zinc-insulin once daily before breakfast. She was feeling well until one day at 3 A.M. she was found by her sister in convulsions and in coma. She could not take anything by mouth. A few minutes after 20 cc. of 50 per cent glucose solution was administered intravenously she was out of coma. She was then given about 40 gm. of carbohydrate by mouth. Her urine remained sugar free. In spite of the fact that the following morning she was given 12 U of insulin less and her diet was divided in frequent feedings, she developed a similar, but less serious spell of insulin coma

CHART 1. CASE 1. A. C., MALE, AGE 39. DIABETES FOR 12 YEARS.

Date 1936	Glycosuria am	n	pm	Blood Sugar F	N	PM	E	Nt	Insulin am	n	pm	Type of Insulin	Remarks, Diet
1/1–2/6													
1/20	0	0	0		179				24	—	22	RI	C180-P100-F100-Cal.-2020
2/4	0	0	0			156			22	6	22		
2/7–3/27													
2/26	+	0	+		97				24	—	*34*		
27	0	0	+	128					24	—	*40*	RI	
28	0	0	+	R		R			24	—	*36*	and	
3/11	0	0	2−		SR				26	—	*30*	PPI	sl. sore throat
20	0	0	+		SR				20	—	*22*		
3/28–5/19													
4/4	0	0	+		96				*24*	—	*28*		
7	0	0	0		166				*24*	—	*28*		
9	0	0	+			132			*26*	—	*30*	PPI	Frequent feedings
16	0	0	0		96				*28*	—	*30*		
24	+	2−	2−	SR	143				*30*	—	*30*		
5/8	0	0	0	R					*28*	—	*30*		
13	0	0	0			181		R	*28*	—	*28*		
19	0	+	0	SR					*28*	—	*30*		
5/20–6/31													
5/29	0	+	0			R			*32*	—	18		
6/3	0	+	2−		215				*34*	—	20		
4	0	0	0					SR	*34*	—	20	PPI	
9	0	+	+		238				*40*	—	18	and	Frequent
23	0	0	+		260				*44*	—	18	RI	feedings
25	0	0	0				64	R	*46*	—	18		
26	0	4−	4−						*44*	—	18		
28	0	0	0				SR		*44*	—	18		
30	+	3+	3+		300				*44*	—	12		
7/1–8/17													
7/7	0	+	+		163				*38*	—	10		
9	0	0	+	SR					*38*	—	10		
13	0	0	+		180				*38*	—	10		
17	0	0	0		270			SR	*38*	—	10		
18	+	4+	4+		250	235			*38*	—	8	PCI	
21	0	+	+						*40*	—	10	and	
27	0	0	0	R					*40*	—	10	RI	Frequent
29	0	+	+		174	R			*40*	—	8		feedings
30	0	+	+			380		R	*42*	—	8		
8/4	0	0	0			165			*42*	—	8		
5	0	+	0		156				*42*	—	6		
12	0	0	0						*44*	—	6		
16	0	0	0						*44*	—	6		
8/18–9/9													
8/23	0	0	+					R	*50*	—	6		
24	0	0	0			211			*50*	—	4	PPI	
26	+	+	0				230		*50*	—	4	and	Frequent
9/1	0	0	+		129				*52*	—	6	RI	feedings
3	0	2+	2+	168					*52*	—	6		
8	0	+	+		250				*54*	—	4		
9/10–9/15													
9/10	0	0	0			85			*56*	—	—		Frequent
11	0	0	0						*60*	—	—	PPI	feedings
14	0	0	0		132				*60*	—	—		
15	0	0	0		109								
9/16–9/20													
9/16	0	0	0						*48*	—	—	PZI	Frequent
18	0	0	0		120	140			*48*	—	—	unmixed	feedings
20	0	0	+						*48*	—	—		

CHART 1. (*Continued*)

Date 1936	Glycosuria am	n	pm	Blood Sugar F	N	PM	E	Nt	Insulin am	n	pm	Type of Insulin	Remarks, Diet
9/20–9/27													
9/21	0	0	0				R		*48*	—	—	PCI	Frequent feedings
26	0	+	0		112	135			*48*	—	—		
27	0	+	0		134				*48*	—	—		
1937													
1/1–1/15													
1/6	0	0	+						*48*	—	—		3 mealsand
9	0	0	0					R	*48*	—	—	PZI	bed time
12	0	+	+		200				*46*	—	—	mixed	lunch
13	0	0	0			R			*46*	—	—		
15	0	0	+						*46*	—	—		

F, fasting. *N*, 10 a.m. to 2 p.m. *PM*, 2 p.m. to 6 p.m. *E*, 6 p.m. to 10 p.m. *Nt*, 10 p.m. to 8 a.m. *R*, reaction. *RI*, regular insulin. *PPI*, plain protamine insulin. *PCI*, protamine-calcium-insulin. *PZI*, protamine-zinc-insulin. *CI*, crystalline insulin (Stearns).

the next night. I then reduced her insulin to 40 U. Since November 1936 her diabetes has been controlled on about 30 U of protamine-zinc-insulin once daily. Her diet was divided in 5 feedings.

This case illustrates that severe reactions, even insulin coma, may occur with protamine-zinc-insulin; that these reactions can occur during the night, without premonitory symptoms and that drastic reductions in the insulin dosage

CHART 2. CASE 2. H.B., AGE 32. DIABETES FOR 4 YEARS.

Date 1936	Glycosuria am	n	pm	Blood Sugar F	N	PM	Nt	Insulin am	pm	Type of Insulin	Remarks, Diet
9/13	4+	4+	4+					15	15		C150-P80-F60-Cal.-1460, divided in 3 meals
14	4+	4+	4+			202		60	20&*40*		
15	3+	3+	3+					60	20&*60*		
16							R	60	*60*	RI	
17			2+					40	*52*	and PZI	
18							R	48	*52*	unmixed	
19						206		48	*40*		
20	0		0					*56*	40		
21		0				119	R	*68*	20		
22	0	0						*76*	20		
23			0					*80*	12		
9/24	0	0	0					*80*	—		
25	0	0	0				R	*80*	—		
26	0	0	0				R	*76*	—	PZI	6 feedings, (3 regular meals) and 3 small feedings)
27	0	0	0			R	R	*76*	—	unmixed	
28	0	0	0	R		200		*68*	—		
30	0	0	0			159		*68*	—		
10/10	0	0	0				VSR[1]	*68*	—		
11	0	0	0				VSR[2]	*56*	—		
12	0	0	0					*40*	—		
13	3+	2+	2+		104	92		*40*	—		
15	0	0	0			131		*40*	—		
19	0	0	0								
11/23–1/31											
12/16	0	0	0			77		*32*	—	PZI	
1/26	0	0	0			73		*30*	—	mixed	

See Chart 1 for explanation of symbols.

[1] Very severe reaction; insulin coma at 3 a.m.; 20 cc. of glucose given intravenously and 40 gm. of carbohydrate by mouth.

[2] Very severe reaction; insulin coma at 5 a.m.; 60 gm. of carbohydrate given by mouth.

may be necessary in some cases after they have been regulated for a short while. The latter is important in the cases which are uncontrolled at the time protamine-insulin treatment is started. See chart 2.

Case 3. W. B., a male, age 51, was first seen at the Diabetic Clinic in 1934 when he presented all the classical symptoms of diabetes mellitus, undiagnosed for about 1 year. He was regulated on a diet of C-210, P-80, F-110, cal.-2150, with about 16 U of regular insulin daily. He was soon found to be 'insulin sensitive' and required 2 injections a day in spite of the fact that 16 U was sufficient to control his diabetes. Even on a morning dose of 8 U he would be on the verge of a reaction during the forenoon. Still in the afternoon his urine would show sugar.

Protamine insulin was instituted on March 21, 1936. In spite of the fact that the patient was sensitive to protamine insulin as well as to the regular, he was able to take the total amount of insulin he required in a single dose.

The reaction with the regular insulin usually occurred in the forenoon, while with the various protamines it would occur at any time of the day, mostly in the afternoon and during the night. The reactions were mild and easily relieved with about 10 gm. of carbohydrate, or by his meal. The highest blood sugar with the protamine insulin was found in the forenoon. To counterbalance this, part of the carbohydrate from his breakfast was transferred to the evening. There was no great difference in this case between the effect of the calcium or zinc preparations.

Crystalline insulin gave results somewhat similar to those with the zinc and calcium preparations. It was difficult, however, to keep the urine completely sugar free the entire 24 hours. Reactions with crystalline insulin occurred in the forenoon and in the afternoon, but not during the night.

This case was 'insulin sensitive' to all the known types of insulin. In spite of this, protamine insulin was the preparation on which the patient did best, requiring a single dose daily, and being sugar free during the entire 24 hours. See chart 3.

Case 4. B. S., a male, age 18, was first found to have diabetes mellitus in March 1936, when he was admitted to the hospital in diabetic acidosis. He was regulated on a diet of C-210, P-110, F-100, cal.-2180, with insulin, 24 U before breakfast, 22 U before lunch, and 24 U before supper. His insulin dosage was gradually reduced to 16 U before breakfast, 20 U before lunch and 16 U before supper. On Sept. 1, 1936 he was started in the Out-Patient-Department on protamine-zinc-insulin. While trying to adjust him on different regimes it was noticed that his insulin requirement increased markedly. In spite of a reduction in his diet to C-180, P-100, F-100, Cal.-2020, and the use of 102 U of regular insulin plus 40 U of protamine-zinc-insulin a day, he developed acidosis and was again admitted to the hospital. This time he was discharged on a diet of C-150, P-90, F-80, cal.-1680, with 46 U of protamine-zinc-insulin before breakfast, and 20 U of the regular before lunch and supper. Within the following 2 months he was gradually regulated on a single dose of protamine-zinc-insulin given before breakfast. Although previously suspected, the patient now admitted marked indiscretion in his diet at the time he presented the difficulties in the clearing up of his glycosuria.

This case illustrates the importance of diet in the regulation of diabetes. It shows that indiscretion in diet may bring about acidosis during the use of protamine-zinc-insulin in combination with regular insulin in large doses. See chart 4.

CHART 3. CASE 3. W. B., MALE, AGE 51. DIABETES FOR 12 YEARS.

Date 1936	Glycosuria am	n	pm	Blood Sugar F	N	PM	E	Nt	Insulin am	pm	Type of Insulin	Remarks, Diet[1]
1/1–3/30												C-210,
3/20	+	+	2+	258					8	8		P-80, F-110
23	+	+	2+		R		260		16	—	RI	Cal. 2150
26	0	+	2+		118				16	—		
3/31–6/15												
4/22	0	0	0		90				*30*	—		[1]
5/14	0	0	0			77		R	*26*	—		
26	0	0	0		160				*26*	—	PPI	
6/11	0	0	0			78			*26*	—		
12	0	0	0	98					*26*	—		
15	0	0	0			53			*26*	—		
6/16–7/9												
6/24	0	0	0		R	R			*14*	—		
25	0	0	0			78	122		*14*	—	PCI	
27	0	+	0			118			*14*	—		
30	0	0	0	88					*14*	—		
7/7	0	0	0		139				*14*	—		
7/10–7/28												
7/15	0	0	0		154				*18*	—		
18	0	0	0		R	R			*20*	—	PPI	
20	0	0	0			165			*16*	—		
28	0	0	0		163	R			*22*	—		
7/29–8/18												
8/6	0	0	0		170			R	*16*	—	PZI	[2]
18	0	0	0			R		R	*18*	—	unmixed	
8/19–9/3												
8/26	0	0	0				134		*18*	—	PPI	
9/1	0	0	0		180	125			*20*	—		
2	0	0	0	63					*22*	—		
9/4–10/27												
9/8	0	0	±		R				*16*	—		
10	0	±	0		111 R	65			*18*	—	CI	[3]
13	0	±	±	152	82				*16*	—		
26	0	±	±		52 R	78			*18*	—		
10/2	0	0	±	132					*16*	—		
19	0	0	0		R				*18*	—		
10/28–12/10												
10/29	0	0	0		132				*16*	—	PZI	
11/4	0	0	0			79		R	*18*	—	mixed	
12	0	0	0		114				*18*	—		
12/10	0	0	0			R		R	*18*	—		

See Chart 1 for explanation of symbols.
[1] On the verge of a reaction during the entire 24 hours.
[2] On the verge of a reaction before each meal.
[3] Never a reaction during the night.

DISCUSSION

Protamine-zinc-insulin, as used today, is the final product of a great deal of experimentation in the Laboratory and in the Clinic. Hagedorn (1) was the first to observe that the addition of protamine to regular insulin greatly retarded its action. It forms, by precipitation, an insulin compound, the solubility of which is the lowest at the pH of body fluid. Hagedorn and his co-workers have observed that their protamine insulin was of value in only a

small group of diabetics, namely those with fluctuating blood sugars. In such cases they have recommended the use of protamine insulin in the evening in combination with regular insulin in the morning. They have also suggested a special adjustment of the diet. On this regime they were able to keep the blood sugar of severe diabetics on a fairly normal level during the entire 24 hours.

Simultaneously with the development of protamine insulin some observers such as Bertrand and Macheboeuf (2), Scott and Fisher (3) and a number of others (4) found that various metals such as cobalt, nickel, calcium and

CHART 4. CASE 4. B.S., MALE, AGE 18. DIABETES SINCE MARCH 1936.

Date 1936	Urine AM	N	PM	Blood Sugar F	N	PM	E	Insulin am	n	pm	Type of Insulin	Remarks, Diet
6/16	+	0	0		134	71		24	22	24	RI	C210, P110, F100,
8/25	0	2+	0			77		16	20	16		Cal. 2180
9/1	3+	0	0					14	12	*30*		
10/13	3+	4+	4+					18	10	*30*		Diet same.
10/20	3+	3+	3+					*44*[1] 14	—	6	RI & PZI unmixed	3+ acetone; blood CO_2-43.
10/22	3+	3+	3+					*48* 24	20	26		
10/27	2+	3+	3+					*40* 26	20	40		C180, P100, F100, Cal. 2020.
11/3	3+	3+	3+					*40* 30	20	36		1+ acetone. Acidosis.
11/12	3+	3+	3+					*40* 34	28	40		Blood CO_2-31. Admitted Hosp.
12/8	0	0	0			94		*46*	20	20		Discharged.
12/31 1937	0	0	0		95			*46*	20	24	PZI mixed & RI	C150, P90, F80, Cal. 1680.
1/7	0	0	0		118			*56*	20	—		
1/12	0	0	0	R	134			*60*	12	—		Carbohydrate of diet
1/26	0	0	0		158		R	*68*	4	—		divided in 1/5, 2/5, 2/5 and some at bedtime.
1/27	0	0	0			263		*68*	—	—	PZI	
2/2	0	0	0		122			*68*	—	—	mixed	

See Chart 1 for explanation of symbols.
[1] Beginning Oct. 20 until Nov. 12 patient was receiving in the morning protamine zinc insulin and regular insulin.

zinc have a beneficial effect on the biological action of insulin, Scott and Fisher (3) believe that zinc plays the most important role among the above mentioned metals.

With the addition of small doses of calcium or zinc to the protamine insulin its stability has been improved, and its hypoglycemic action has been prolonged. As a result the number of diabetics who could benefit by it has greatly increased. After some experimentation it was decided that the zinc preparation has some advantages over the calcium preparation.

The first product used in these studies consisted of plain protamine insulin. This was soon changed to protamine insulin containing calcium or zinc,

distributed unmixed. Since Oct. 1936 my patients have been receiving protamine-zinc-insulin, mixed. A few patients have received crystalline insulin in intervals for experimental purposes.

Protamine-zinc-insulin has been used alone and in combination with regular insulin. When used alone the best results were obtained with a single dose, usually before breakfast. Occasionally a patient preferred to take it before lunch or supper. In some cases it was necessary to combine the protamine-zinc-insulin with regular, temporarily or for an indefinite length of time. In such cases the regular insulin was given in a small dose, usually at the same time with the protamine-zinc-insulin or occasionally later during the day.

With the marked indiscretion in diet, and during various complications, such as a cold, infection, acidosis or any surgical interference in patients who have been receiving protamine-zinc-insulin, the addition of regular insulin was almost a rule. At times the dose of the then required regular insulin was large. With the improvement of the complicating condition, however, the regular insulin could often be discontinued.

In case of coma or in any other diabetic emergency, when quick acting insulin was necessary, regular insulin was always given first. Protamine-zinc-insulin was only used to supplement and prolong the action of the regular insulin.

Special attention had to be given to the rearrangement of the diet in order to avoid insulin reactions. The total of the diets prescribed, however, remained unchanged. Most of them contained 150 to 200 gm. of carbohydrate, 80 to 100 gm. of protein and 60 to 100 gm. of fat. The adjustment of the diet depended on the insulin regime. When protamine insulin was used in the morning, the patient was given more of the slower absorbing carbohydrates such as bread for his supper and a small lunch at bedtime, as part of his diet. 'Insulin sensitive' patients required some carbohydrate between meals and on rising. Some patients were able to get along with 3 meals a day. In such cases it was best to divide the carbohydrate of the diet in 1/5 for breakfast, 2/5 for lunch and 2/5 for supper. When protamine insulin was used in the evening, the bedtime lunch could be omitted.

Insulin reactions occurred frequently at the beginning of these studies, although not as frequent as with regular insulin. The reaction from the protamine insulin is at times severe, more lasting and frequently recurs. It requires larger amounts of carbohydrate for relief. Occasionally it may start with nausea and vomiting. In such a case it may present quite a diagnostic problem since nausea and vomiting may occur in acidosis or during a gastro-intestinal upset or appendicitis. Glucose for intravenous use should always be on hand.

Rabinowitch et al. (5) have stressed the fact that with protamine insulin the blood sugar can be kept for many hours below normal level without any signs of a reaction. While a real explanation of it is still wanting, I do not consider the absence of signs or symptoms of a reaction in the presence of an abnormally low blood sugar as an advantage in the treatment of diabetes. Prolonged hypoglycemia is known to be injurious in many ways to the patient. It may affect the circulatory as well as the nervous system. It would seem there-

fore that a prolonged hypoglycemia even without symptoms, is not a favorable state to look for. Our aim should be to keep the blood sugar of the patient within normal limits, as much as possible, avoiding hypoglycemia as well as hyperglycemia. Frequent blood sugar determinations are therefore indicated until the diabetes is regulated.

SUMMARY

The effect of the various kinds of protamine insulin, namely, plain protamine insulin, protamine insulin with calcium or zinc, distributed unmixed, as well as protamine-zinc-insulin mixed, as now on the market, has been studied in over 100 cases of diabetes of varying severity and of various ages. Crystalline insulin was tried in a few patients for the purpose of comparison. A few cases have been presented for illustration.

Protamine-zinc-insulin helps to reduce the number of injections in many uncomplicated cases to a single dose daily. In fact in some cases the results are best when given in a single dose. In diabetic coma, as well as in other diabetic complications regular insulin should always be used first.

The diet plays an important role in treatment with protamine-zinc-insulin. Its carbohydrate content should not as a rule exceed 200 gm. and the total amount of calories should be restricted. The carbohydrate of the diet should be divided so as to avoid insulin reactions.

Insulin reactions may be severe. They should be looked for in the latter part of the day or during the night when protamine-zinc-insulin is used in the morning. Individualization of every case is necessary in regard to the time of administration of the protamine-zinc-insulin, the number of units, and the various ways of its use in combination with regular insulin, as well as in regard to diet.

April 7, 1937 case 4 was readmitted to the hospital because of severe diabetic acidosis (blood CO_2-24), which he developed, while on 68 U of protamine-zinc-insulin daily and on a diet of C-150, P-90, F-80, cal.-1680. The cause of his acidosis is not known.

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FATAL FUNCTIONAL UTERINE BLEEDING

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Fatal uterine hemorrhage is rare, except when it is due to complications of pregnancy or to corporeal and cervical tumors; it is then usually associated with some hematological dyscrasia. Payant (1), Van Vugt (2) and Frazer (3) each described such an occurrence in young women which was attributed to thrombocytopenic purpura. Payant's patient showed symptoms and signs of intraperitoneal hemorrhage for which a ruptured Graafian follicle was considered responsible. In none of these cases was a study of the ovaries or endometrium possible since neither curettement, laparotomy nor necropsy was made. Kermauner (4) reported the death of a 15-year-old girl with myeloid leukemia on the 12th day of her first menstrual period. An involuting corpus luteum was observed in one ovary at necropsy; the endometrium was not described. Fleischmann (5) detailed the necropsy findings in the case of a young woman, 24 years of age, dying from excessive uterine bleeding in association with pernicious anemia. There were observed atrophy of the thyroid, hyperplasia of the thymus, reduction in the chief cells of the hypophysis, genital hypoplasia and large ovaries, one of which contained a corpus luteum. Fischer (6) collected from the literature several other reports of fatal uterine hemorrhage attributed to blood dyscrasias.

Plaut (7) and Munzescheimer (8) each described a patient with fatal uterine hemorrhage unassociated with any hematological factors. Plaut's patient was 12 years of age and died during the course of her first menstrual period. The ovaries and uterus were judged normal by pelvic examination. Curettage yielded a small amount of tissue which was not examined microscopically. Necropsy was refused. Plaut refers to 2 similar patients one described by Pinkus, 13 years of age, and another by Dann, 14 years of age. Munzescheimer's patient, aged 17 years, had had irregular episodes of amenorrhea and metrorrhagia since menarche. A small amount of endometrium was obtained by curettage, microscopic examination of which showed only small fragments of tissue with scattered interval glands in a very dense stroma. At necropsy the ovaries were slightly cystic and contained no corpora lutea.

It is interesting that in none of these cases of fatal functional uterine hemorrhage was there described what is termed 'endometrial hyperplasia' but rather endometria of hypoplastic or atrophic type. Apparently in none of these cases was the pituitary studied and in only the one of Munzescheimer were necropsy findings recorded. In view of these facts, it seemed advisable to report our case with an interpretation of the apparent etiologic factors.

CASE REPORT

M.S., a single colored girl, aged 15 years, was referred to Duke University Hospital, May 13, 1935, for treatment of excessive uterine bleeding. She was moribund when she arrived at the dispensary and died on the way to the ward, despite emergency stimulative measures and while arrangements were being made for transfusion.

History. From her mother it was learned that, since menarche 2 years previously, there had been periods of amenorrhea interspersed with episodes of bleeding which on several occasions lasted for a month and assumed alarming proportions. The last bleeding had begun about one month prior to admission and had persisted until 3 days before entry. The blood loss was excessive; 5 to 6 napkins a day were required. Weakness from this blood loss had confined the patient to bed for 3 weeks. The patient gave no history of purpuric skin manifestations or of bleeding from mucous membranes.

Physical Examination. The patient was a semi-comatose, extremely anemic, well-developed colored girl. Temperature (rectal) was 38.4° C., pulse 120 per minute, respiration 24 per minute and blood pressure 140/60. Secondary sexual development was normal. The thyroid was not enlarged. A loud blowing systolic murmur was heard over the entire precordium. The heart was not enlarged. Inspection showed external genitalia and distribution of pubic hair to be normal. A taut hymenal ring did not allow unidigital vaginal examination. Recto-abdominal palpation identified uterus, cervix and ovaries of normal size and in normal position. No abnormal adnexal masses were encountered.

Laboratory Findings. Hemoglobin was 12 per cent (Sahli), erythrocytes 850,000 and leukocytes 25,900. The differential count showed polymorphonuclear neutrophilic leukocytes 87 per cent, large lymphocytes 1 per cent, and small lymphocytes 12 per cent. In the smear a few nucleated erythrocytes were observed and there was marked central achromia. Urinalysis gave normal findings. The sedimentation rate was 11 mm. in 30 minutes and 30 mm. in 60 minutes. Blood chemistry values included calcium 8.8 and phosphorus 8.3 mg. per cent; refractive index was 1.3480. Unfortunately we were not able to check this abnormally high phosphorous content and have no explanation for it.

Pathologic Report. The necropsy was performed 40 hours after death. The body had been kept in a cooled compartment. There were no external abnormalities. The breasts were well developed. On opening the body all of the tissues were pale. The pleural and peritoneal cavities contained an excessive amount of clear amber fluid (about 500 cc. in each pleural cavity, and 600 cc. in the peritoneal cavities.)

The heart was enlarged weighing 425 gm., and both the right auricle and ventricle were dilated. Both gross and microscopic examination revealed a considerable amount of fat in the myocardium. The lungs showed nothing abnormal except a number of small subpleural hemorrhages. The liver was swollen. Microscopically there was observed a marked accumulation of fat around the central vein as well as some necrosis of the liver cells in this region. The bone marrow taken from the shafts of femur and humerus and from the vertebra was hyperplastic.

The thyroid was not enlarged. Microscopic sections showed acini with flattened lining cells, some of which contained large amounts of colloid and others showed some epithelial hyperplasia and scanty colloid. There was a pronounced edema of the glottis and epiglottis. Permission to examine the brain was refused.

Each ovary measured approximately 5 x 2.5 x 1.5 cm. (fig. 1). Each contained several follicular cysts averaging 1.5 cm. in diameter. The tunica was smooth and

glistening and there were no ovulational scars. No corpora lutea were present. Serial sections of the ovaries were studied. The germinal epithelium was preserved in only a few places and appeared normal. The cortex was slightly thickened and there was an increase in the stroma. Numerous primordial follicles were present. These were frequently found in clusters. The number observed, however, was slightly less than usually seen at this age. A few early, intermediate and developing follicles were found. The granulosal cells in these had undergone marked cytolytic changes, and showed no evidence of proliferation and no mitotic figures. There was no theca lutein activity and no perifollicular congestion or hemorrhage. No mature follicles were found. The granulosa was fragmentary in some of the follicular cysts (fig. 2, *4*) and absent completely in the majority of them. Various stages of atresia and fibrosis were observed. The chief change was that of fibrosis.

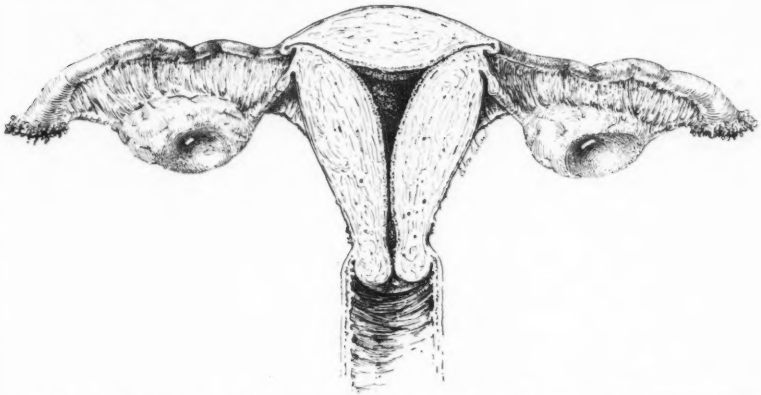

Fig. 1. The ovaries, tubes and uterus at necropsy. Note the smooth glistening tunica of the ovaries, the absence of corpora lutea or ovulational scars and the well developed uterus and cervix.

The uterus was not enlarged and was firm. The endometrium appeared thin and dark red on section. It measured less than 1 mm. in average thickness; the greatest thickness noted was 1.3 mm. Adhering to its anterior surface, was a friable blood clot measuring 4 x 0.5 x 1 cm. Microscopically the stroma of the endometrium appeared dense, the endometrial vessels were dilated widely (fig. 2, *1*) and contained thrombi. Some of the vessels were 0.3 mm. wide. There was definite rhexis of the vessels with hemorrhage. The epithelium was intact in areas (fig. 2, *2*). There was a striking scarcity of glands, only a very occasional small interval one being observed (fig. 2, *3*).

DISCUSSION

As in the other reports of fatal functional uterine bleeding, the endometrium in our case was atrophic rather than hyperplastic. Such an endometrial pattern, as one of us (9) has emphasized, is encountered not uncommonly in association with adolescent bleeding and usually indicates clinically a severe grade of ovarian failure.

The assumption that such a pattern is related to a hypoestrin phase seems compatible with the ovarian and endometrial findings at necropsy. These findings include marked degenerative changes of the granulosa and striking paucity of endometrial glands. The normal somatic and sexual development

of the patient is not irreconcilable with such a point of view since the estrin requirement for such a process is materially less than that necessary for endometrial proliferation. Relative crucial variations in this low estrin level may be considered as responsible for the bleeding and non-bleeding phases.

Changes in the proliferation, tonicity and integrity of the components of the vascular tree of the endometrium are thought to have been the direct cause of the fatal hemorrhage. The widely dilated vessels of this endometrium, with obvious rhexis, constitute the most striking finding in the study. These

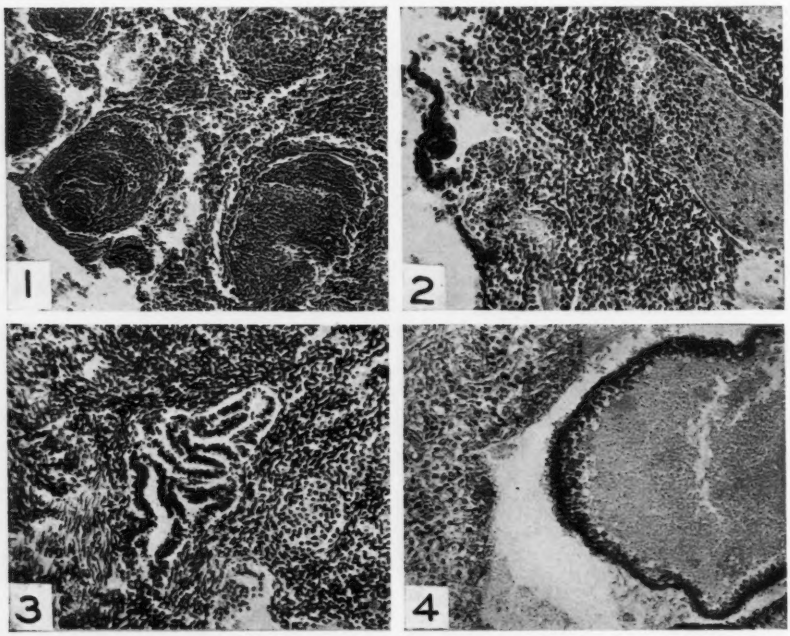

Fig. 2. Photomicrographs of endometrium and ovaries. X94. *1).* Widely dilated endometrial vessels with rhexis. *2).* Strip of endometrium covered with epithelium and containing no glands. *3).* One of the few sections of endometrium in which glands were observed. *4).* The wall of a follicular cyst showing degeneration of granulosa.

vascular phenomena are considered causally related to the low estrin level. No myopathic factors are apparent except, perhaps, that of uterine atonia due to estrin deficiency. There is no evidence to be adduced as to the level of pituitary function in this case.

SUMMARY

A study of fatal uterine hemorrhage in a 15-year-old girl is described and the necropsy findings detailed. The endometrium was atrophic being characterized by a paucity of glands and widely dilated vessels. The ovaries showed cysts with degenerated granulosa and contained no corpora lutea. Critical estrin deficiency with impairment of vascular proliferation, tonicity and integrity is suggested as the responsible mechanism.

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CACHEXIA HYPOPHYSEOPRIVA (SIMMONDS' DISEASE)

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Cachexia hypophyseopriva or Simmonds' disease is due to atrophy of the anterior lobe of the pituitary gland. The onset of this disease is insidious, usually in the third or fourth decade, and occurs chiefly in women, the picture being characterized by marked emaciation, precocious senescence, gastro-intestinal distress, loss of the secondary sexual characteristics and intense debility. In addition, hypothermia, bradycardia accompanied by hypotension, a reduced metabolic rate and diminished salt and water excretion are present, with death terminating the syndrome. There may be marked somnolence, diminished mental powers and a tendency to melancholia (1, 2).

Anterior pituitary extracts have been used in the treatment of this condition with improvement as reported by Herman (3) and Dunn (4). In this paper we wish to report a typical case of Simmons' disease in which treatment with anterior pituitary extracts was not beneficial.

CASE REPORT

K. S., an unmarried Jewish woman of 28 years, was admitted to the Pennsylvania Hospital on Nov. 10, 1935, complaining of nausea, vomiting, backache, flatulence and loss of weight. Her father died at the age of 58 of carcinoma of the face, the mother died through an accident at the age of 57, four siblings died in infancy of unknown causes; one brother, 40 years of age, was living and well. She had convulsions in infancy, measles in childhood and frequent attacks of tonsillitis for which a tonsillectomy was performed in 1929.

The patient felt well prior to 1929, then she became very nervous and developed bad eating habits, followed by periods of nausea and vomiting and severe abdominal pain. In 1930, following the death of her father and mother, which circumstance changed her economic status from one of comparative ease to one of almost poverty, these symptoms became worse. She complained of loss of weight (35 lbs in 5 years), easy fatigue, excessive thirst, abnormal appetite, vomiting, backache, urgency and burning on urination, tingling sensations throughout her body, occipital headaches and marked vertigo. In 1932, all of her lower teeth and most of the upper ones were extracted after becoming loose and infected. In June, 1935, on the last of 3 occasions when her ankles were swollen, she was unable to put on her shoes. Menstruation began at the age of 16, occurred 2 to 3 times a year and lasted for 6 days; she had amenorrhoea since 1928. During this period, she had several admissions to 6 different hospitals.

Physical examination showed an extremely emaciated, dehydrated, poorly developed white female, who gave her age as 28 but who appeared at least 40 years of age. She was apprehensive and nervous. Her temperature was usually about 97°, pulse 68, respiration 24, blood pressure 96/78. She was 5 ft. 4 in. tall and weighed 80 lbs. The body framework was small with wasted musculature hanging on projecting bones. Her eyes were prominent, her head appeared large with abnormal

amounts of hair on the face; the skin was pale, ichthiotic, cold, dry and inelastic; the nails were short, brittle and raised from the matrix. She had no lower teeth and a partial upper plate with 3 remaining upper teeth present. Her neck was thin with a few palpable submaxillary nodes. The chest was of the asthenic type with complete atrophy or lack of development of the breasts and absence of axillary hair. The chest was clear to percussion and auscultation. There was no cardiac enlargement; rhythm was regular and the sounds were of fair quality; no murmurs were noted. The abdomen was thin and flat, the liver edge was palpable 3 cm. below the costal margin in the right mid-clavicular line. The spleen and kidneys were not palpable. There were no other palpable organs or masses. Peristalsis was active. A pelvic examination by Dr. C. B. Lull revealed fairly normal external genitalia, scanty pubic hair, hypertrophy of the labia minora and infantile uterus and adnexa (by

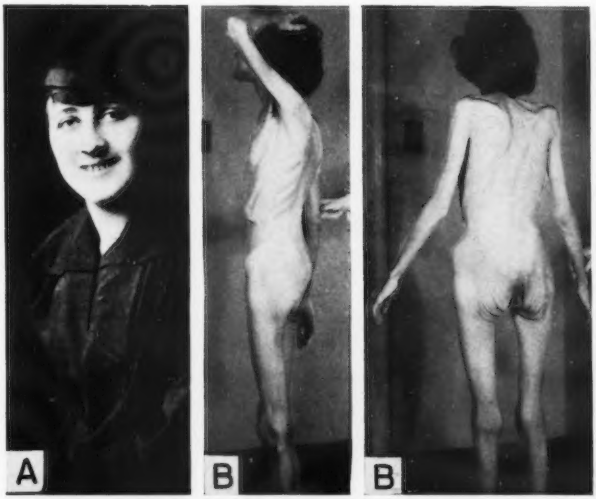

Fig. 1. *A*, Photograph of the patient at the age of 18. *B*, at the age of 28.

rectal examination). No tumor of the ovary was found. The reflexes were present but diminished; sense of position, vibration, pain and touch were normal. There was some loss of muscular power and some sclerosis of the peripheral vessels noted. The dorsalis pedis and posterior tibial pulse were barely perceptible.

The blood picture was not remarkable, with no evidence of eosinophilia. Urinalyses showed an occasional trace of albumin and sugar. The blood Wasserman and Kahn reactions were negative. Gastric analysis showed the absence of free HCl until after the administration of histamine, when hypoacidity was demonstrated. The B.M.R. varied from —20 on admission to —11 per cent 4 months later. The specific dynamic action of a protein meal was tested with results as follows: B.M.R., taken at 8:15 A.M., —33 per cent pulse 64; at 9 A.M., 500 gm. of meat was given; second B.M.R., at 11 A.M., —25 per cent, pulse 86, an increase of 8 points in the metabolic rate as compared to the normal increase of 20. Insulin tolerance was markedly reduced, 1U producing a reaction with sweating, syncope and nausea, and a blood sugar of 39 mg. 1.5 hours after the administration of the insulin. The glucose tolerance test revealed definitely increased tolerance on admission, but fol-

lowing the treatment with anterior pituitary extract, the curve of blood sugar values became more normal.

GLUCOSE TOLERANCE VALUES

Date	F	½ hr.	1 hr.	2 hr.	3 hr.	4 hr.	5 hr.	6 hr.	7 hr.
11/18/35	80	79	64	137	119	80	83	68	75
1/6/36	75	108	162	133	108	88			

The Volhard water excretion test demonstrated a definite reduction in urinary output, and a lessened salt elimination was revealed by the chloride excretion test. Stool examinations gave negative findings. Eye grounds and visual fields were normal.

X-ray examinations, made by Dr. Paul Bishop, revealed the pituitary fossa normal in size and shape. There was no evidence of bone erosion. There was considerable rarefaction and trabeculation of the long bones of the upper extremity. These conditions were also present in both fibulae; there was a suggestion of trabeculation in the femurs and tibia. The remaining upper teeth were also x-rayed and showed no evidence of abscess formation, but there was considerable alveolar absorption. The gall bladder was faintly visualized and filled with a great many small, round shadows of non-opaque stones. Electrocardiographic findings were within normal limits.

The neurological consultation by Dr. B. J. Alpers, the psychiatric by Dr. H. B. Palmer, the surgical by Dr. Charles Mitchell, the eye by Dr. Warren Reese and the skin by Dr. A. D. King disclosed no other abnormalties, and it was felt that the symptoms could be explained entirely by the endocrine disturbance.

The treatment that was instituted consisted at first of anterior pituitary extract, Squibb, beginning with a dose of 1 cc., or 10 R.U., every other day and increasing to 5 cc., or 50 R.U., a total of 2070 U being thus administered. Following this, a trial was made with the growth hormone of the anterior pituitary, as isolated by Collip and prepared by Ayerst, McKenna and Marrison. The patient had 7 doses of 5 cc. or 250 R.U. each, a total of 1750 U. The maturity factor of the anterior pituitary, also isolated by Collip, was then tried, 3 doses of 250 R.U. each being given. The treatment was discontinued because the patient became dissatisfied and wished to leave the hospital. She was, therefore, discharged on May 11, 1936.

At times there was a slight gain in weight and we felt encouraged, but at the end of our therapy there was very little change in the patient that could be measured except a slight improvement in the basal metabolic rate and a sugar tolerance curve a little closer to normal.

SUMMARY

A case is presented of cachexia hypophyseopriva in an unmarried Jewish woman of 28 years. Clinical and laboratory findings show this to be a typical case although it is not proven by autopsy. Treatment was carried out with anterior pituitary extract (Squibb), the growth hormone of the anterior pituitary, and the maturity factor of the anterior pituitary, a total of 4570 R.U. being administered. Failure of this therapy to obtain beneficial results is recorded.

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CURRENT ENDOCRINE LITERATURE

COLLABORATORS

Fuller Albright
I. Andrussier
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ADVANCE ABSTRACTS

Acceleration of the rate of passage of fertilized ova through the fallopian tubes of mice by massive injections of an estrogenic substance. Burdick, H. O., and R. Whitney. To appear in this Journal.

Subcutaneous injections of 100 to 500 R.U. of the estrogenic substance progynon-B caused an acceleration of the rate of passage of fertilized ova through the fallopian tubes of mice. Ova were frequently recovered in the uterus, even in the 2-celled stage, 30 to 40 hours after vaginal plug formation.

Effects of long-continued 'night-lighting' on hypophysectomized female ferrets and those with optic nerves cut. Bissonnette, T. M. To appear in this Journal.

Mature females ferrets were night-lighted. One group was hypophysectomized, another had the optic nerves cut and a third served as unoperated controls. From Jan. 15 to July 7, 1934 the 2 latter groups and 4 daughters of the group with the optic nerves cut were given various light regimes at different times until 1937, such that they served as experimentals and controls upon themselves. Hypophysectomy abolished sexual and hair cycles up to 405 days, with and without experimental lighting. Cutting the optic nerves freed both cycles from photic control. Estrus occurred July to December instead of March to August, and earlier in the 2nd and 3rd summers. Double cataract in one female with optic nerve cut suspended both cycles for 2 years. Young females may be brought to estrus 193 days before normal. Estrus, with or without pregnancy or pseudo-pregnancy, in winter, delayed normal estrus the next spring or required more lighting to induce it. Females near the end of one estrus, unmated, or entering anestrus, can be brought back to estrus with little or incomplete vulval shrinking. Two estrous seasons a year may be induced. Females, like males, became refractory to photic sexual stimulation after a time and required greater stimulus to reinduce estrus.

The biological effects of thymectomy. Accruing retardation in growth and development in successive generations of thymectomized rats. Einhorn, N. H., and L. G. Rowntree. To appear in this Journal.

Thymectomy in successive generations of parent rats caused retardation in growth and development of the offspring. This retardation is amplified as thymectomy is carried through succeeding generations to the 6th. Of a total of 108 litters comprising the test rats of the 2nd, 3rd, 4th, 5th and 6th generations, one 2nd-generation litter consisting of 2 males showed a rate of growth greater than that of the operative controls. In the remaining 107 litters the rate of growth fell below that of the controls. The retardation in growth is most marked during the prepubertal period, reaching a maximum average retardation of 20% at 30 days of age in the 6th generation. At 70 days of age, however, each succeeding generation of the thymectomy strain falls further below the weight of controls than the preceding generation. Both parents must be thymectomized in order to produce retardation in the rate of growth and development of the offspring.

Complete and incomplete estrogenic hormones, arising from different sites in the rat's ovary. Freed, S. C., and S. Soskin. To appear in this Journal.

The ovary of the rat secretes at least 2 estrogenic hormones, one from the theca and the other from the granulosa. The estrin of the theca is incomplete. It does not induce endometrial proliferation. The follicular estrin is complete, in a relative sense. These results aid in the interpretation of previous experimental work, and the possibility of applying these results to the interpretation of clinical disorders in the humans is suggested.

Treatment of sexual underdevelopment with synthetic male hormone substance. Hamilton, J. B. To appear in this Journal.

Synthetic male hormone substances were administered to a 27-year old hypogonadal male, physiologically castrate from birth, who provided an excellent subject for evaluation of the effect of these materials. The hypogonadism, cryptorchidism, impotence, hot flushes, migraine, and mental attitude were treated with some degree of success. Brief discussion is given of the relation of these results in this clear-cut case to the subject of male hormone treatment, with especial reference to cryptorchidism, hypogonadism, impotence, menopausal symptoms, migraine and acne. The possible influence of male hormone upon the voice, the area of pigmented skin under the eye and the mental attitude is mentioned.

Differential diagnosis of pseudocryptorchidism and true cryptorchidism. Hamilton, J. B., and G. Hubert. To appear in this Journal.

A technique is described of direct application of heat to scrotum, inguinum and perineum for the purpose of obtaining relaxation of the muscles which produce spastic retention. This method, plus the precautions, general procedures and observations enumerated, permits differential diagnosis of intermittent and continuous retention of the testicle. Employment of this technique reveals the large percentage of pseudocryptorchid testes that are considered true cryptorchidism by usual tests, even by physicians experienced in the handling of children. Of the 16 cases referred to the authors by pediatricians all but 6 presented a condition of spastic retraction. The high incidence of pseudocryptorchidism masquerading as true cryptorchidism leads one to question the large percentages of 'spontaneous descent' in supposedly true cryptorchidism. Further, it may partially explain the discrepancy in the results of various authors in the treatment of cryptorchidism with endocrine preparations.

Ovaries secrete male hormone. III. Temperature control of male hormone output by grafted ovaries. Hill, R. T. To appear in this Journal.

Twelve castrated male mice received grafts of ovaries subcutaneously near the tip of the ear. Six of the host males were put in a chamber of 33°C. and 6 were kept in a surrounding temperature of 22°C. All of the animals in the lower temperature developed completely normal seminal vesicles and prostates at 55 to 156 days after grafting. In direct contrast, the accessories of the 6 grafted castrated males at the higher temperature (33°C.) showed no response at all. It is concluded that temperature can control and accentuate the male hormone output of a graft ovary.

Testis hormone in relation to age. Hooker, C. W. To appear in this Journal.

From a series of bulls ranging in age from 1 month to 15 years the testes and 24-hour urines were extracted for male hormone. Assays of the testis extracts show that the hormone content increases slightly but progressively to the age of about 2 years, when there is a sharp increase which is continued to 5 years. After 5 years the hormone content gradually decreases. Since puberty is attended by no sharp change in the rate of hormone production by the testes, the hypothesis is advanced that at this age the tissues acquire a capacity to respond to a quantity of hormone previously ineffective.

A comparison of the ketosis developed during fasting by obese patients and normal subjects. MacKay, E. M., and J. W. Sherrill. To appear in this Journal.

New experiments along with data from the literature are used for a comparison of the extent of fasting ketosis in obese and non-obese subjects which have hitherto been believed to be similar. Patients who were apparently only overweight and might be said to have simple obesity always developed as large and usually a greater ketosis than non-obese controls. A number of obese patients had a fasting ketosis much smaller in extent than is ever seen in a well-nourished normal individual. Their examination suggested some endocrine disturbance

in every case. The results suggest that the method is worthy of further use as a possible means of classifying obesity upon a physiological basis.

Studies on the physiology of lactation. VII. Lactation in thyroidectomized rats and guinea pigs. Nelson, W. O., and C. E. Tobin. To appear in this Journal.

Fifteen rats thyroidectomized during the last half of pregnancy continued to term, delivered normal litters, and suckled them successfully. Ten rats thyroidectomized prior to the incidence of pregnancy delivered and suckled successfully one or more litters, 17 in all. Six female guinea pigs were thyroidectomized during the last 10 days of pregnancy. In each instance parturition and lactation occurred in a normal fashion. Four male and 2 female guinea pigs were injected with estrin for 28 days. In each instance the thyroid was removed and the injections of estrin withdrawn. All animals began lactating within 3 days. Two male guinea pigs were thyroidectomized and injected with estrin for 28 days. In each instance lactation occurred following the cessation of estrin treatment. Seven guinea pigs which had received injections of estrin for 30 days were hypophysectomized. Treatment with lactogenic hormone and orally administered desiccated thyroid failed to initiate lactation. Two of these animals were subsequently induced to lactate by substituting cortical hormone for the thyroid substance. Three lactating guinea pigs were hypophysectomized and given lactogenic hormone and thyroid substance. Lactation ceased within 2 days. This evidence shows that mammary gland development and lactation may occur in the absence of the thyroid gland. Furthermore, the thyroid hormone when given with lactogenic hormone failed to initiate or maintain lactation in hypophysectomized animals.

The bitterling fish response to male sex hormones. Owen, S. E. To appear in this Journal.

It is difficult to break ovipositor dormancy in female bitterling fish by treatment with the sex hormones. The nuptial coloration in male bitterling fish may be produced rather easily by means of several endocrine preparations. In the search for biological test objects for endocrine preparations, it appears that more knowledge of the chromatophore reactions and ovipositor changes in fish is essential before these may be used for specific endocrine assay purposes. The normal factors associated with the spawning season changes must be studied; among these are hydrogen-ion concentrations, temperature levels, radiant energy factors and others.

Epithelial growth caused by stimulation with various smear methods as demonstrated by mitotic stasis with colchicine. Rogers, P. V., and E. Allen. To appear in this Journal.

Using the colchicine technique a comparative study of the degree of epithelial stimulation resulting from the taking of vaginal smears was made on 15 experimental rats. The controls consisted of a number of castrate animals receiving only colchicine and a series of rats injected with colchicine after being brought into estrus with theelin. Rats carefully smeared with a moist toothpick showed very little cell proliferation when compared with those smeared with a cotton swab. The greatest stimulation was present about 32 hours after first using the cotton swab. At the end of 80 hours there was an increase in the thickness of the epithelium but no sign of cornification.

Study of the Gilfillen-Gregg skin test for pregnancy. Saglik, S., and E. Scipiades, Jr. To appear in this Journal.

The intradermal skin test of Gilfillen and Gregg proved 100% negative in pregnant and non-pregnant monkeys (16) as well as in 4 female rats, 2 pregnant and 2 non-pregnant. Injection of prolan or estrone did not sensitize the monkey to the reagent (follutein Squibb). A small control series of 19 non-pregnant and 23 pregnant women demonstrated inferior reliability of the test as compared with the Aschheim-Zondek or Friedman test.

The use of estrin in the treatment of eclampsia. Shute, E. To appear in this Journal.

Two detailed histories illustrate our present views. The first is strongly suggestive of impending convulsions, although one can never say with certainty that such a patient must have had convulsions if left alone. In each case the hormonal situation was the same—a high excretion of prolan and a low or normal blood content of estrogenic substance. In each case the administration of estrogenic substance very soon showed itself in both the blood serum and the onset of labor. Under the influence of estrin and sedative therapy the patients did not grow worse and the convulsive patient, indeed, rapidly improved objectively in spite of some rise of blood pressure. Convulsions ceased in the second case as estrin control was

established, and this can scarcely be ascribed to the sedative therapy, as delivery occurred 13½ hours after the last dose of sedative and long after her drowsiness had largely disappeared. Estrin control was established more readily than would be suspected, if the change in blood reaction and the onset of labor pains are any indication of that. Allowing as much time for the second dose of progynon to take effect as was taken by the first dose brings us to the time of the sudden haemorrhage and the development of unusually severe pains, a picture strongly suggestive of abruptio placentae, and if so, confirming our theory of the relationship of excess of estrogenic substance in the blood to the onset of abruptio placentae. This certainly suggests that estrin therapy, during pregnancy at least, should be used very cautiously and in small dosage. It is difficult to induce labor in eclamptic patients if they are not already in labor when the convulsions develop and they are notoriously liable to infection. The estrin type of therapy may suggest an alternative and safer method of handling these cases.

Some observations on the gonadotropic hormones of pregnancy. Tenney, B., Jr., and F. Parker, Jr. To appear in this Journal.

In early pregnancy the gonadotropic hormone recovered from the urine is nearly entirely of placental origin as shown by its reaction on hypophysectomized rats and its complete precipitation by alcohol. In normal cases at term this APL substance has practically disappeared from the urine and a pituitary-stimulating substance is present which will act on immature but not hypophysectomized rats. In toxemias at or near term there is an increased amount of APL which can be recovered from the urine with a decrease or absence of the pituitary-stimulating substance present in normal cases.

Comparative action of testosterone compounds, of estrone and of combinations of testosterone compounds and estrone on the anterior hypophysis. Wolfe, J. M., and J. B. Hamilton. To appear in this Journal.

Thirteen immature rats received 10 daily injections of 500 γ of testosterone-propionate. Eleven litter-mates served as controls. Such injections induced degranulation of the basophiles in the anterior hypophysis. No constant or noteworthy changes were observed in either the eosinophiles or the chromophobes. Mitotic activity was not increased in the injected rats; the pituitary weights were unaltered from those of the controls. Three groups of 10 female rats received, respectively, 10 daily injections of 500 γ of testosterone-acetate, 10 daily injections of estrone, and 10 daily injections of 500 γ each of these 2 factors. Injections of the male factor simultaneously with estrone, partially suppressed the characteristic reaction of the anterior lobe to injections of estrone, i.e., marked weight increase, complete degranulation of the basophiles, degranulation of numerous eosinophiles and a marked decrease in the percentages of the chromophilic cells and an increase in those of the chromophobes. A similar suppression of the action of estrone on the anterior hypophysis by injection of male hormone simultaneously with estrone, was also obtained in similar groups of castrated female rats and normal males.

The functional efficiency of transplanted adrenal cortical tissue. Wyman, L. C., and Caroline tum Suden. To appear in this Journal.

The lethal intraperitoneal dose of histamine for adrenalectomized rats of an inbred white strain having intramuscular transplants of adrenal cortical tissue was variable but averaged about one-half that for normal rats, and 4 or 5 times that for rats having no cortical tissue. Homoplastic transplants afforded protection against histamine equal to that given by autoplastic grafts. Administration of a cortical extract (Eschatin) increased the percentage of survivors and extended the survival period of the fatal cases, but did not restore normal resistance. The results of blood-pressure experiments were consistent with those of the histamine tolerance tests.

ADRENALS

Studies of the suprarenal glands of dogs. I. Comparison of the weights of suprarenal glands of mature and immature male and female dogs. Baker, D. B., Am. J. Anat. **60:** 231. 1937.

A study of the weights of the suprarenals of 1250 dogs showed that the glands of the females in diestrus were larger than those of the mature males of the same body weight. The glands of the immature animals represented approximately 85 to 88% of the weight of those of the mature dogs. With each pound increase in body weight there was an approximate

average increase of 0.03 gm. in the weight of the pair of glands of the mature male and 0.04 gm. in the weight of the pair of suprarenals in the female in diestrus. Six left suprarenals were sectioned serially with the freezing microtome. Every fifth section was stained with Scharlach R and hemalum and projected. The volume of the medulla was found to vary from 8.9 to 18.6% of the gland. The ratio of the medulla to the cortex was approximately 1 to 5 in mature males and females in diestrus. The glomerulosa represented approximately 24% of the cortex. Between 52.8 and 67.2% of the cortex was fasciculatis. The shapes of the suprarenals were different and definite enough for them to be recognized as right or left.—Author's Abst., Wistar Bib. Service.

The adrenal gland in new-born mammals. Bourne, G., Proc. Linn. Soc. N.S.W. **61:** 221. 1936.

The adrenal glands of the following animals were examined: 2 new-born specimens of zebra; 1 new-born Polar bear; 1 young seal (a few weeks old); 1 late foetus of a Dolphin; 1 ten-inch pouch embryo of the tree kangaroo; 3 pouch embryos of ground kangaroo and 1 young female kangaroo. All these animals except the zebras were museum specimens so that intricate cellular detail could not be given. The adrenals of the zebras were treated to demonstrate histological detail, lipoids, Golgi apparatus, mitochondria and vitamin C. The adrenals of the zebras showed a thin outer rim of cells and a voluminous medullary part. There was a striking zona glomerulosa which fact constituted a difference from the adrenals of the young ungulates examined by Hill. The adrenals of the young marsupials have been found to pass through a stage comparable with that of the adrenals of the non-primate eutherian mammals. This is true also for the dolphin. A young seal possessed an adrenal in which a definite differentiated cortex was present, thus constituting a difference from the adrenals of members of the Carnivora vera examined by Hill.—G. B.

Serum electrolytes and mineral metabolism in a case of Addison's disease, with observations on the use of suprarenal cortical extract (Eschatin). Chou, S. K., K. C. Chen, S. H. Liu and S. S. Fang, Chinese M. J. **50:** 1013. 1936.

The authors give results of a complete investigation of a case of Addison's disease with pulmonary tuberculosis, in a Chinese male 38 years of age. The patient exhibited the typical Addisonian syndrome, asthenia, anorexia, pigmentation, and hypotension. It was found that the treatment of the patient with cortical extract gave at first a slight subjective and objective improvement. Later treatment was ineffective. Investigation of the serum electrolytes during the progress of the disease showed an increase in total base, sodium and chloride. The blood volume was low. The preliminary treatment with eschatin gave rise to some alteration in the serum electrolytes but the second treatment produced no change. The authors reach the conclusion that replacement therapy in Addison's disease is still in an unsatisfactory position. Either the available commercial preparations are too dilute or they deteriorate rapidly.—G. B.

The innervation of the adrenal glands. Hollinshead, W. H., J. Comp. Neurol. **64:** 449. 1936.

Section of the splanchnic nerves in the cat has produced degeneration of a varying percentage of the nerve fibers in the adrenal gland. Section of the roots of the lower thoracic nerves, or of the lower thoracic and upper lumbar nerves, has likewise produced degeneration of nerves in the adrenal in both cats and dogs. After section of both splanchnics, and removal of the upper lumbar sympathetic chain, the nerves in the medulla have disappeared, except for a few fine fibers which apparently supply blood vessels. The majority of the nerve fibers in the adrenal gland are therefore preglanglionic in nature. Very few nerve cells were found in the glands of the animals used, and the plexus among the chromaffin cells had degenerated in the operated cases. The preganglionic fibers apparently end among the medullary cells without the interposition of postganglionic neurones, as suggested by Elliott.—Author's Abst., Wistar Bib. Service.

Sympathico-adrenal-hepatic mechanism and blood potassium (Mecanismo simpático-adrenalino-hepático y potasio sanguíneo). Houssay, B. A., A. D. Marenzi, and Rebeca Gerschman, Rev. Soc. argent. biol. **12:** 434. 1936.

In experiments on chloralosed dogs direct stimulation of the hepatic nerves produced an increase in blood K, due to discharge of K from the liver (increase in suprahepatic veins). Stimulation of the peripheral end of the nerves of the kidney, pancreas and spleen did not

produce an increase in blood K. Strong stimulation of the sciatic nerve produced a small but significant increase in blood K, even after removal of the adrenals and the liver. Stimulation of the splanchnic nerves or of the nerve centers (electrical, stimulation of the medulla, asphyxia, anemia due to vagal stimulation of the heart, hemorrhage) produced an increase in blood potassium due to adrenalin discharge (principal factor) and to direct stimulation of the hepatic nerves (accessory factor). Drugs that stimulate the adrenal medulla directly (nicotin, acetylcholin, tetramethylammonium) produced an increase in blood K, but not after the adrenals or the adrenal medulla had been removed. Agents acting on the adrenals produced their effect by means of adrenalin discharge, not by liberation of K (no increase of K in adrenal venous blood). Adrenalin acted directly on the liver. Ephedrin, pitressin and tyramin acted directly on the liver and their activity was less or absent after the adrenal secretion was suppressed. Marked asphyxia and profuse hemorrhage also produced a slight, inconstant, direct effect on the liver.—J. T. L.

The action of potassium chloride and of calcium chloride on the adrenals of the cat. Katz, G., and Gertrude Katz, J. Pharmacol. & Exper. Therap. **59:** 284. 1937.

Two methods were used for determining the discharge of epinephrin, Feldberg's and the reaction of the nictitating membrane. The cats were anesthetized with dial or chloralose and then eviscerated. Potassium chloride, 2.5 to 5 mg., intravenously, usually had no effect on either the nictitating membrane or the blood pressure but this amount injected into the coeliac artery caused a rise in blood pressure and a retraction of the nictitating membrane. Calcium chloride, 1 to 5 mg., into the coeliac artery, caused similar effects to those produced by potassium chloride. The effects could be increased by cocaine and abolished by the removal of the adrenals.—E. L.

Innervation of the aortic paraganglion of the abdomen in mammals (Zur Innervation des Paraganglion aorticum abdominale bei einigen Säugetieren). Kofmann, V., Ztschr. f. Anat. u. Entwcklngsgesch. **105:** 305. 1935.

From a study of 50 dogs, 20 cats, 14 rabbits and 10 humans (from 7 month fetus to 2 yrs. of age) it is concluded that the chromaffin tissue of the organ of Zuckerkandl is as well supplied with nerves as the adrenal medulla.—A. T. R.

Chemical identification of the 'hormone of the adrenal cortex active on muscle' (Die chemische Identifizierung des "Muskelwirksamen Nebennierenrindenhormons"). Lang, K., and B. Stuber, Biochem. Ztschr. **284:** 256. 1936.

The authors confirmed the observation of others that the adrenal cortex contains a lipoid-soluble substance which delays the onset of fatigue in isolated frog muscle. The test object was the isolated frog gastrocnemius. The active substance was isolated and identified as an ester of a fatty acid. It was, furthermore, found that various synthetic esters have the same action and the authors concluded, therefore, that the assumption of a cortical hormone, active on muscle and different from cortin, is unnecessary.—E. Bozler.

On the chemical nature of the substances of the adrenal cortex reducing the blood lipoids. Second communication on the adrenal cortex (Ueber die chemische Natur der die Blutlipoide senkenden Substanzen der Nebennierenrinde. 2. Mitt. über die Nebennierenrinde). Lang, K., and B. Stuber, Biochem. Ztschr. **284:** 270. 1936.

The observation of previous authors that acetone and ether extracts of the adrenal cortex lower blood cholesterin and phosphatids in rabbits was confirmed. However an effect similar to that of extracts was obtained by injecting esters of fatty acids. The esters of acids with a small number of C-atoms lower chiefly the phosphatids, those with greater number of C-atoms influence chiefly blood cholesterin. The authors consider the assumption of hormones of the adrenal cortex controlling the lipoid metabolism as unnecessary.—E. Bozler.

ENDOCRINE GENERAL

Changes in the lateral-line organs during the life of the newt, Triturus viridescens. A consideration of the endocrine factors involved in the maintenance of differentiation. Dawson, A. B., J. Exper. Zoöl. **74:** 221. 1936.

In the larva, the lateral-line organs are superficial and are exposed on the surface. During the terrestrial stage the organs lie deeper in the dermis and are covered by an overgrowth of epidermal cells. Both sensory and supporting cells are partially dedifferentiated. At the close

of the terrestrial phase the regressive organs are reconstituted. The communication with the free surface is regained and the number of epithelial elements is increased by mitosis of both sensory and supporting cells. Cupulae, sometimes vertically striated appear irregularly on the surface of the organs. The maintenance of complete differentiation in this stage appears to depend on the thyroid gland which is controlled by the anterior pituitary. Hypophysectomy and thyroidectomy caused a marked regression of the sense organs while anterior pituitary stimulation resulted in hypertrophy and hyperplasia of the sensory and supporting cells. The cupulae and cupular secretion are also under endocrine influence. Following hypophysectomy or thyroidectomy the cupular secretion appeared as globules distributed throughout the distal portion of the supporting cells, while after pituitary stimulation the secretion was accumulated in the dilated tips of the mantle and interstitial cells. During depressed thyroid activity or early in the absence of the thyroid or pituitary gland the secretion was viscid and when liberated the products of individual cells tended to retain their identity producing the characteristic vertical striations of the cupula.—Author's Abst., Wistar Bib. Service.

The choline-esterase activity of blood serum from two cases of myasthenia gravis. Hicks, C. S., and Margaret E. MacKay, Australian J. Exper. Biol. & M. Sc. 14: 275. 1936.

A severe case of myasthenia gravis and an early case of the same disease have been studied continuously for 11 months and 4 months, respectively, for cholin-esterase activity of the blood serum. Simultaneous hydrolytic and synthetic experiments were used in the investigation. Using the perfused frog's heart, the isolated frog's rectus muscle, and the surviving intestine, evidence has been obtained of increased cholin-esterase activity of the serum of the myasthenic patient, measured by both hydrolysis and resynthesis of acetylcholin. At no time has the patient's cholin-esterase activity failed to exceed that of the normal, and it has approached the normal value only when symptoms had almost disappeared. The severity of the symptoms was closely followed by the esterase activity of the serum. It is concluded that the increased esterase in the patient's blood (which is not quantitatively related to severity of symptoms) is an overflow from muscle tissues where its production is excessive and where consequently the rate of accumulation of adequate acetylcholin is reduced sufficiently to impair transmission of the nervous impulse. Prostigmin (Roche) and eserin both inhibited the synthetic as well as the hydrolytic action of cholin-esterase, but in certain synthesis experiments eserin lost its inhibiting effect. Prostigmin was not subject to this variation of action. Normal blood serum from the same and from different individuals did not show any variations in cholin-esterase content when estimated by means of isolated organs.—Authors' Summary.

The influence of insulin administration on the cytology of the hypophysis and several other endocrine glands (Vlianie khronicheskoi insulinizatsii na gistostrukturu gipofiza i nekotorykh drugykh endokrinnykh jelez). Kan, K. Z, Problemy endokrinologii 5: 5. 1936.

Eighteen guinea pigs were injected with insulin subcutaneously—0.5 U per 2 kg. daily. They were killed after 5, 9, 15 and 26 days. There was noted hypertrophy and hyperplasia of the eosinophile cells of the pituitary, increased vascularity of the islands of Langerhans and stimulation of external secretory activity of pancreatic parenchyma, hypertrophy of the adrenal cortex and a stimulation of the secretory activity of the medulla. The effect on the thyroid gland was not consistent. The pituitary was fixed in mercury bichloride, mounted in paraffin and stained with hematoxylin and Mallory technique. Sections from all parts of the anterior lobe were observed and 2000 to 3000 cells counted. In 4 control animals 65 to 70% were chromophobe, 25 to 30% eosinophile and less than 10% basophile. In the injected animals the portion of eosinophiles was increased to 42.7%, the relative proportion of chromophobe cells diminished, while the basophiles remained unaltered. The author regards his results as further proof of the chromophobe origin of the eosinophile cells, and evidence that the carbohydrate-influencing fraction of the pituitary is contained in the eosinophile cell.—B. N. Tager.

Hypophysis-thyroid-gonad relationship. Leonard, S. L., Proc. Soc. Exper. Biol. & Med. 34: 599. 1936.

Extracts of urine from a castrate woman and from a man with testicular tumor were more potent in stimulating the ovaries and uteri of thyroidectomized immature rats than of normal immature rats. These extracts exhibited no thyrotropic activity when tested in young guinea pigs or hypophysectomized rats. Similar results were obtained with a follicle-stimulating extract prepared from anterior pituitary, which was likewise relatively free of thyrotropic activity.

The author cites the different behavior of P.U. which is equally effective in normal and thyroidectomized animals.—M. H. F.

GONADS

Effect of prolonged administration of estrone on the skin of white rats (Influencia de la administración prolongada de oestrona sobre la piel y anexos de la rata blanca). Arguello, R. A., Rev. Soc. argent. de biol. **12:** 468. 1936.

Young white rats of both sexes, weighing 15 to 60 gm., received 12 to 40 units of estrone daily subcutaneously for 56 to 102 days. The dose of estrone was regulated so as to maintain the females in continuous estrus. The final weight of treated animals was significantly lower than that of litter mate controls. Histologic sections of the skin showed: marked hyperplasia of the corneal layer; atrophy and degeneration of the epithelial layer; decrease and abnormal distribution of the pigment; considerable decrease of the adipose tissues; great decrease of the hair follicles in the superficial layers and almost complete disappearance in the deeper layers of the dermis. These alterations were more marked in the skin of the back than in that of the abdomen. The possible role of the pituitary and thyroid is discussed.—J. T. L.

Sexual responsiveness and temporally related physiological events during pregnancy in the rhesus monkey. Ball, Josephine, Anat. Rec. **67:** 507. 1937.

The sexual responsiveness of 3 rhesus monkeys was measured before and after conception. A drop in responsiveness after the pre-ovulatory rise at conception was followed by another rise the following month as if the ovulation cycles were continuing. After this, scores remained practically at zero in the 2 normal cases. The third animal aborted after a third cyclic rise in responsiveness. These results are correlated with other indicators of the shifting of endocrine control while the embryo is establishing its relation to the maternal organism, namely, changes in vaginal desquamation, rise and fall of urinary prolan, slight regression of the corpus luteum.—Author's Abst., Wistar Bib. Service.

The fate of mouse ova tube-locked by injections of oestrogenic substances. Burdick, H. O., R. Whitney and G. Pincus, Anat. Rec. **67:** 513. 1937.

Normally the mouse ovum enters the uterus 72 to 96 hours after copulation in late morula and blastocyst stages. However, if estrogenic substances in sufficient quantity (7.5 R.U. per day) are injected into female mice after mating the fertilized ova remain in the oviducts. It was found that estrogenic substances do not affect early cleavage stages of the developing tubal ova. The normal round blastocyst stage, in which the trophoblast cells are in contact with the zona pellucida, is usually attained. Tube-locked ova ordinarily begin to degenerate at about the time when the zona pellucida is lost at the end of the round blastocyst stage. The tubal environment does not favor the continual growth of the blastocyst; collapse and fragmentation occur, and the ova disintegrate completely by 7 days after copulation.—Authors' Abst., Wistar Bib. Service.

Effect of small doses of estrin upon ovarian and vaginal cycles of mature rats. Button, L. L., and C. I. Miller, Proc. Soc. Exper. Biol. & Med. **34:** 835. 1936.

The daily injection of ¼ to 2 R.U. of estrin into mature rats for periods of 3 to 13 weeks, led to irregularities in the cycles, consisting of either prolonged estrus or diestrus, and reduced the incidence of ovulation as judged by the number of corpora lutea present at autopsy. The injections also led to a reduction of ovarian weight.—M. H. F.

Inhibition of menstruation by crystalline progesterone. Corner, G. W., and W. M. Allen, Proc. Soc. Exper. Biol. & Med. **34:** 723. 1936.

The daily injection of 1 mg. of crystalline progesterone into monkeys, beginning one week before the expected onset of the next menses, prevented bleeding during the course of the injections which were continued for about 2 weeks after the expected menses. Bleeding occurred 2 to 4 days after cessation of treatment.—M. H. F.

Ovarian hormones and thyroid function (Ovarialhormone und Schilddrüsenfunktion). De Amilibia, E., M. M. Mendizábal and J. Botella-Llusiá, Klin. Wchnschr. **15:** 1001, 1936.

The injection of 200 units of folliculin into male rats produced a stimulation of the thyroid as judged by these investigators from the histological picture. Extracts of corpus luteum had no effect.—M. H. F.

The sexual cycle of the chimpanzee. Elder, J. H., and R. M. Yerkes, Anat. Rec. **67**: 119. 1936.

Daily observations for more than 5 years on a colony of captive chimpanzees show that the sexual cycle, while similar in essential features to that of other primates, is about a week longer than in man and monkey. Records show the average cycle to be 36 days with a median and modal length of 35 days. Periodic, external, genital swelling, a spectacular although typical feature of the chimpanzee cycle, provides a valuable means of determining the sexual status of the female at any given time. Evidence on ovulation time, obtained by means of controlled matings and other criteria, indicates that the period of greatest fertility in the chimpanzee varies according to the length of the cycle; that for individuals with short cycles (33 to 37 days) it occurs between the 16th and 20th days following menstruation but as late as the 23rd or 24th day in individuals with cycles of more than 40 days. The beginning of detumescence is more constant in its temporal relation with the end of the cycle than the beginning—Authors' Abst., Wistar Bib. Service.

Sexual dimorphism of the pelvis of the mouse, the effect of estrogenic hormones upon the pelvis and upon the development of scrotal hernias. Gardner, W. U., Am. J. Anat. **59**: 459. 1936.

A sexual dimorphism occurs in the pelves of mice. In male mice the pubes converge medially to form the cartilaginous symphysis. The pubes of female mice underwent a slight decalcification following sexual maturity and the symphysis became ligamentous. The pubes of primiparous animals were separated by interpubic ligaments which increased in length during later pregnancies. The shape of the innominate bones of mature females differed from that of the males. Males bearing ovarian grafts showed a partial resorption of the pubes and occasionally interpubic ligament proliferation, particularly castrate males. Extensive decalcification of the pubes and proliferation of interpubic ligaments occurred in male or female mice receiving adequate amounts of different estrogenic chemicals. The age of the mice at the time the injections were started and the sex were associated with variations in the extent of the pelvic changes. The female-shaped pelvis was not observed in any of the treated male mice and was considered to be determined by other than ovarian influences. Bilateral or unilateral scrotal hernias occurred in many of the male mice. The modification of the pelvic musculature coincident with the pelvic changes and the presence of testes of approximately normal size were always associated with the occurrence of hernias.—Authors' Abst., Wistar Bib. Service.

Simulation of birth-mechanism in experimentally monotocous and oöphorectomized rats. Haterius, H. O., Proc. Soc. Exper. Biol. & Med. **35**: 197. 1936.

Although castrate rats carrying one fetus and several intact placentae will continue to carry the pregnancy to term, or beyond, labor does not set in and the young must be removed surgically. In the present study, rats so prepared, were injected with increasing doses of estrin starting on the 15th day of gestation, at the time of the ovariectomy. Although the response varried with the dosage administered, and from animal to animal with the same dosage, it was possible to cause evacuation of the uterine contents by estrin administration, and in several instances viable young were delivered.—M. H. F.

A consideration of the nutritive state in the metabolism of women during pregnancy. Hummel, Frances C., Helen A. Hunscher, Mary F. Bates, Priscilla Bonner, Icie G. Macy and J. A. Johnson, J. Nutrition **13**: 263. 1937.

Uninterrupted metabolic balances of an 18-year-old primapara living under highly controlled environmental conditions and eating a constant diet during the final 65 days of gestation showed a mean daily storage of nitrogen, phosphorus, chlorine, sulfur, calcium, magnesium, sodium and potassium of 1.32, 0.27, —0.17, 0.27, 0.71, 0.06, 0.11, 0.01 gm., respectively. The medical history of this woman showed her to be underweight and of an unsatisfactory nutritional state for 6 years immediately preceding conception. In comparing these figures with similar ones from the literature on 2 healthy women during the same interval of gestation it is shown that nutritive state and physiological conditions other than gravidity influence the maternal storage of chemical elements. Such continuous metabolic studies reveal significant variations among individuals under similar physiological states should be considered when using the metabolic balance for determining and interpreting dietary requirements.—Authors' Abst., Wistar Bib. Service.

The nomenclature of "male hormones." Advisory Committee on the Nomenclature of Endocrine Principles. J.A.M.A. **107:** 210. 1936.

A chronological summary is given of the recent evolution of knowledge concerning the various chemical bodies having a masculinizing influence. These are referred to as hormones though no proof is offered that any is genuinely such. It is suggested that all masculinizing substances be designated, generically, as 'androgens.' Of the androgens, that derived from the urine is 'androsterone,' $C_{19}H_{30}O_2$; its melting point is 178 C. Another crystalline body derived from testicular substance is 'testosterone,' $C_{19}H_{28}O_2$; its melting point is 154 C; it has several times the potency of androsterone, by capon test. Also derived from urine is 'dehydro-androsterone,' $C_{19}H_{28}O_2$, melting at 148 C., and approximately 1/5 as potent as androsterone. Several other androsterones are described and the structural relationships among them indicated. All are assayed in terms of comb-growth response in capons, that of 100 micrograms of androsterone serving as the international unit. It is emphasized, however, that the capon response is not necessarily an accurate index of response in mammals.—R. G. H.

The reversal by progestin of responses of the non-pregnant uterus of the cat. Kennard, J. H., Am. J. Physiol. **118:** 190. 1937.

The uteri of mature spayed cats prepared by progestin administration displayed a reversal in response to stimulation of the hypogastric nerve, and adrenin injection. Progestin in the minimal amounts effective in reversing nerve responses, failed to alter response to adrenin. With adequate dosage reversal occurred first in nerve response and secondarily, much later if at all, in adrenin response. For a short period the uterus reacted to nerve stimulation by contraction and to adrenin administration by relaxation. The author suggests, on the basis of Cannon and Rosenblueth's hypothesis, that progestin is effective in converting the hypothetical inhibitory substance (I) to excitatory substance (E) within the muscle cells. Innervated cells would contain (E), non-innervated cells (I). Since the latter predominate numerically intravenous adrenin, affecting all cells simultaneously, would form an excess of sympathin (I), inducing relaxation. Nerve stimulation, on the contrary, liberating adrenin primarily within innervated cells containing predominantly (E), would result in the formation of sympathin (E) and consequently would induce contraction.—H. O. Haterius.

The effect of the testicular hormone upon the fertilizing ability of sperm (Über die Einwirkung des Testikelhormones auf die Befruchtungsfähigkeit des Spermas). Koch, W., Klin. Wchnschr. **15:** 629. 1936.

The injection of ½ bird unit of male hormone (Erugon) twice weekly from January to March into 2 cocks increased the fertilizing ability of their sperm as evidenced by higher percentage of fertilized eggs (88.6% vs. 43.4%) and greater number of chicks (259 vs. 89) from hens served by these injected cocks than from hens served by 2 normal cocks. It is supposed that the hormone concerned is different from the one affecting secondary male characters.—M. H. F.

Hormonal modification of the sex ratio in the dog (Über hormonale Beeinflussung des Geschlechtes beim Hunde). Koch, W., Klin. Wchnschr. **15:** 1135. 1936.

Feeding 35 to 80 M.U. of estrin daily to bitches for 6 to 12 before coitus increased the percentage of males in the offspring.—M. H. F.

Concentration and purification of the gonadotropic substance in urine of ovariectomized and post-menopausal women. Levin, L., and H. H. Tyndale, Proc. Soc. Exper. Biol. & Med. **34:** 516. 1936.

A method involving precipitation of the activity by tannic acid is described, together with the technique of further purification.—M. H. F.

Effect of testis on the mammary gland. McEuen, C. S., H. Selye and J. B. Collip, Proc. Soc. Exper. Biol. & Med. **35:** 56. 1936.

The normal degree of development of the mammary gland in the male rat is prevented by castration. Moreover, castration of adult males led to regression of the mammary tissue already developed. The injection of 200 gamma of testosterone into immature female rats daily for 7 days, resulted in marked mammary gland proliferation, but did not produce estrus.—M. H. F.

Treatment of menstrual migraine with small doses of gonadotropic extract of pregnancy urine. Moffat, W. M., J.A.M.A. **108:** 612. 1937.

After experimenting with the gonadotropic factor from pregnancy urine in the treatment

of menstrual headache for 2 years the author evolved the following routine: Beginning on the fifth to the seventh day after the onset of the menstrual flow a small dose, usually ranging from 2 to 6 R.U., is given; the dose is slightly increased daily until the tenth day, then rapidly increased, reaching a maximum of from 50 to 125 R.U. on the fourteenth day, after which from 25 to 50 U. is given daily until the onset of the next menstrual period. His results in seventeen selected patients followed over a period of 3 years tend to confirm the hypothesis that a leading factor in the production of menstrual migraine is either an ovarian or an anterior pituitary dysfunction, and probably an ovarian hypofunction or pituitary hyperfunction. All were relieved although some, usually those who had suffered most severely over the longer periods, have been unable to discontinue treatment entirely. These were selected cases, all with a special type of migraine. In only 4 of the 11 cases studied roentgenologically was a normal sella turcica found. To explain the results, the following hypothesis is submitted: These 17 women were all menstruating more or less regularly and therefore could not have had a severe form of pituitary deficiency. Consequently administering gonadotropic substance from pregnancy urine to them was equivalent to the combined therapy of this factor and the 'synergist' from the anterior pituitary, which combination has been shown to produce maximal ovarian stimulation.—Abst., A. M. A. Service.

Effect of estrogenic hormone administration upon nasal mucous membrane of the monkey (Macaca mulatta). Mortimer, H., R. P. Wright, C. Bachman and J. B. Collip, Proc. Soc. Exper. Biol. & Med. **34:** 535. 1936.

The injection of estrogenic material produced reddening of the nasal mucosa of monkeys synchronously with the skin changes in the sex-skin, nipples, and face and back. The effects were readily produced in normal and castrate females, immature females and normal adult males. In untreated females peaks of nasal reddening occur at 28-day intervals, at the premenstrual period, and is most marked in the autumn months.—M. H. F.

Inhibition of oestrus and of the vaginal response to oestrone by testosterone. Robson, J. M., Proc. Soc. Exper. Biol. & Med. **35:** 49. 1936.

When regularly cyclic mice were injected with 0.1 mg. of testosterone twice daily, estrus was abolished for the 2-week period of injection. When castrate mice were simultaneously injected with 0.4 mg. of testosterone and with sufficient estrone to induce estrous smears in 50% of control castrates, the estrous reaction was inhibited.—M. H. F.

Electric potentials in normal, castrate, and theelin treated rats. Rogers, P. V., Proc. Soc. Exper. Biol. & Med. **34:** 644. 1936.

During estrus the vagina is electro-negative to the symphysis pubis by many thousand microvolts when measured by the Burr-Lane vacuum tube microvoltmeter. In normally cyclic rats, this peak is reproduced at each estrus, the potential sharply falling to a much lower level during di-estrus. Castration obliterates these cyclic changes in electro-potentials, and reduces the level far below the estrous peak. The injection of theelin produces a sharp rise coincident with the artificial estrous cornification.—M. H. F.

The chemical estimation of theelin with diazobenzenesulfonic acid. Schmulovitz, M. J., and H. B. Wylie, J. Biol. Chem. **116:** 415. 1936.

A colorimetric procedure for the estimation of theelin is described. The method was based on the coupling of theelin with p-diazobenzenesulfonic acid to give a red azo dye which is compared with a standard solution of B naphthol coupled in the same manner. The reaction appears to be molecularly quantitative, and on this basis 1 mg. of theelin was calculated to equal 2666 R.U.—J. M. L.

Effect of large doses of progesterone in the female rat. Selye, H., J. S. L. Browne and J. B. Collip, Proc. Soc. Exper. Biol. & Med. **34:** 472. 1936.

The daily injection of 4 mg. of progesterone to mature female rats for 12 days caused an abrupt cessation of the estrous cycle without vaginal mucinification. There was some atrophy of the ovary, enlargement of the pituitary, and slight atrophy of the thymus. There was no alteration in the mammary gland.—M. H. F.

Influence of light on ovulation in the fowl. Warren, D. C., and H. M. Scott, J. Exper. Zoöl. **74:** 137. 1936.

This study demonstrated that the restriction of egg laying in the fowl (Gallus domesticus) to the daylight period is a result of the bird's reaction to normal alternation of daylight and

darkness. Continuous artificial light caused the fowl to distribute its laying uniformly over the 24-hour period. A reversal of the periods of light and darkness resulted in laying being restricted to the lighted night period. Since the intervals between layings are usually more than 24 hours, successive eggs of a clutch are laid somewhat later each day. When this results in a bird being due to lay after the middle of the afternoon, the oviposition usually does not occur and a day's rest results. A new series is started with morning laying. The interruption in laying has been found to result from a delay in ovulation, rather than a retardation or interruption of the progress of the egg through the oviduct. Changes in the lighting conditions were not reflected immediately in the fowl's laying, but were expressed after about 60 hours. Thus, the regulating effect of light on laying probably has its influence on the later stages of the maturation of the ovum.—Authors' Abst., Wistar Bib. Service.

Reproductive organs of two mammalian hermaphrodites and their response to injections of pregnant mare serum. Wells, L. J., Anat. Rec. **67:** 233. 1937.

Two immature hermaphroditic ground squirrels (Citellus tridecemlineatus) were discovered at exploratory laparotomy. One animal was sacrificed as untreated control, while the other was injected with pregnant mare serum in daily doses of 25 R.U. for a period of 18 days preceding autopsy. Reproductive tracts were photographed in toto and sectioned serially at 10 μ. Each animal exhibited bilateral ovotestes, scrota, tubuli recti, rete testes, ductuli efferentes, epididymides, ductuli deferentes, vesiculae seminales, ejaculatory ducts, glandulae bulbo-urethrales, ostia tubae and cornua uteri, and unpaired urethra (male), glandula bulbi, penis, corpus uterus, and vagina. These animals are thought to be genetic males in which an ovarian cortex developed and the Mullerian system persisted. Absence of the prostate gland in both hermaphrodites is anatomical evidence suggesting that the prostate gland of normal male ground squirrels may originate from Mullerian ducts. Mare-serum injections induced the formation of spermatozoa, Graafian follicles and corpora lutea. All male and female accessory reproductive organs of the treated hermaphrodite were stimulated.—Authors' Abst., Wistar Bib. Service.

Effects of synthetic androsterone on accessory reproductive organs of the male ground squirrel. Wells, L. J., Proc. Soc. Exper. Biol. & Med. **34:** 525. 1936.

Androsterone injected into non-rutting or castrate male squirrels proved as effective in stimulating male accessories as extracts of male urine, but somewhat less effective than an extract from bull testes. However, the injection of synthetic androsterone stimulated the formation of spermatozoa in immature and non-rutting adult squirrels.—M. H. F.

Nicotine toxicity III. Effect of nicotine-containing diets on the estrus cycle. Wilson, R. H., and F. DeEds, J. Pharmacol. & Exper. Therap. **59:** 260. 1937.

On a diet containing 0.025% nicotine, rats exhibited a prolonged and irregular estrous cycle and on a diet containing 0.05% nicotine, the estrous cycle stopped completely. The decreased food intake of animals on the nicotine-containing diets was responsible for alteration of the estrous cycle.—E. L.

The bill of the sparrow as an indicator for the male sex hormone. II. Structural basis. Witschi, E., and R. P. Woods, J. Exper. Zoöl. **73:** 445. 1936.

Under the influence of the male sex hormone the bill of the sparrow changes from the light brown to a slate blue and later to the black color. The microscopical examination reveals that this change is effected by the activity of melanophores which reside in the deep layer of the epidermis. When stimulated by male sex hormone they secrete melanine granules and inject them by means of long processes into the epidermis cells. In the absence of male hormone these chromatophores persist as uncolored but highly ramified cells and can be found in the light bills of males out of breeding season as well as in those of females and castrates of both sexes. Their continual presence explains the quick response of these bills on injections of male hormone.—Authors' Abst., Wistar Bib. Service.

Gonadotropic hormone in the diagnosis of chorionepithelioma. Zondek, B., J.A.M.A. **108:** 607. 1937.

In the pathologic placenta (hydatidiform mole, chorionepithelioma), the production and excretion of gonadotropic substance may be immensely increased. The hormone appears in greatly increased amounts in the blood, urine and spinal fluid. A diagnosis of hydatidiform mole cannot be considered as established unless, in repeatedly performed examinations, at

least 200,000 M.U. of luteinizing principle is found in the urine and, in addition, a positive luteinizing reaction is obtained from the spinal fluid, perferably diluted. It is necessary to rule out toxemia of pregnancy, as in this condition the luteinizing substance is also excreted in the urine. However, only a follicle-stimulating effect is obtained in spinal fluid. If the pregnancy test still remains positive 6 weeks after the discharge of a hydatidiform mole and if the content of gonadotropic substance in the urine has increased progressively, it suggests a diagnosis of chorionepithelioma, particularly if a positive reaction is also found in the spinal fluid. For confirmation of the diagnosis, exploratory curettage is necessary. When the pregnancy test has become negative after the discharge of the hydatidiform mole and then becomes positive after some time there is either a new pregnancy or a chorionepithelioma. The assay of urine for gonadotropic substance is of importance not only for diagnosis but also for prognosis in chorionepithelioma. If the pregnancy test has become negative following therapeutic measures and if luteinizing substance occurs once more in the urine, this indicates that the malignant process is proceeding. Apparent clinical improvement may often be deceiving in such cases. A considerable reduction in excretion of follicle-stimulating hormone occurring suddenly without therapeutic measures is clinically a threatening sign, although when this occurs following therapy it is usually considered a favorable sign. The presence of 800 M.U. of luteinizing substance per gm. of fresh tissue obtained by currettage must be established before a diagnosis of a malignant condition is made. If 100 M.U. per gm. is found in extrauterine tumors, this indicates that the tumor is a metastasizing or extragenital chorionepithelioma. Chorionepithelioma of the testicle or testicular teratoma with chorionepitheliomatous inclusions lead to a strongly increased production and discharge of follicle-stimulating and luteinizing factors. The anterior pituitary in normal pregnancy as well as in chorionepithelioma and in malignant testicular tumor contains little or no gonadotropic hormone.

HYPOPHYSIS

The demonstration of anterior hypophyseal pancreatropic hormone in the blood and urine under normal and pathological conditions (Über den Nachweis des pankreatropen Hormons des Hypophysenvorderlappens im Blut und Harn unter normalen und krankhaften Bedingungen). Anselmino, K. J., and F. Hoffmann, Klin. Wchnschr. **15:** 999. 1936.

An ultrafiltrate from the sera of normal women contained an activity, in 3 cc. equivalents, which was detectable as similar to that of the pancreatropic hormone of the pituitary when tested for its islet-stimulating capacity on rats (histological). A similar activity was found in an alcohol precipitate representing 3 to 7 cc. of urine from normal individuals. Similar quantities of raw urine gave identical results. The activity found in the urine of diabetics was not discernibly less than normal. The active material is thermolabile, and deteriorates on standing even in the dry form.—M. H. F.

Demonstration of posterior-lobe hormone in the blood by means of the ultrafiltration method (Über den Nachweis von Hypophysenhinter-lappenhormonene im Blut mit Hilfe von Ultrafiltrationsmethoden). Anselmino, K. J., and F. Hoffman, Klin. Wchnschr. **15:** 1750. 1936.

The authors give in detail a simple method of preparing suitable glacial acetic acid collodion membranes of suitable porosity, and also the directions for performing the ultrafiltration of blood without acidification or deproteinisation. With the described technique, it was possible to demonstrate anti-diuretic activity in the ultrafiltrate of 30 cc. of plasma to which 0.45 I.U. of posterior pituitary extract had been added when the ultrafiltrate was injected into rabbits in which a water diuresis had been produced. Similarly, it was possible to demonstrate antidiuretic activity in the ultrafiltrate of blood from a dog into which 5 I.U. of pituitary extract had been injected a few minutes prior to securing the blood sample.—M. H. F.

Extirpation of the pars intermedia of the hypophysis in the young amphibian with subsequent silvery condition and metamorphosis. Atwell, W. J., and E. Holley, J. Exper. Zoöl. **73:** 23. 1936. Abst., Arch. Neurol. & Psychiat. **37:** 671. 1937.

Among Rana sylvatica tadpoles in which removal of the epithelial portion of the hypophysis has been attempted at the tail bud stage, animals occasionally appeared which were silvery but underwent metamorphosis. Similar conditions were produced intentionally by removal of from one-third to two-thirds of the caudal portion of the epithelial lobe of the

hypophysis at the tail bud stage. Study of sections showed that in these animals the hypophysis is located farther rostral than normal, that it has no intimate relation with the neural lobe and that it consists of a normally differentiated anterior lobe and usually also of the pars tuberalis. The pars intermedia is lacking. The thyroid gland, the adrenal cortex and the gonads do not exhibit the atrophy characteristic of the completely hypophysectomized animal. It is concluded that the silvery condition or pigmentary reversal occurs because of the lack of the pars intermedia of the hypophysis and that metamorphosis occurs because the anterior lobe proper, which is present, is sufficient to induce adequate stimulation of the thyroid gland.

The effect of route of administration on the bioassay of prolactin. Bates, R. W., and O. Riddle, Proc. Soc. Exper. Biol. & Med. **34:** 847. 1936.

Using pigeons 6 to 7 weeks of age, it was found that the subcutaneous route was 5 times as efficient as the intramuscular route and 11 times as efficient as the intravenous for the injection of prolactin. It was also found possible to detect 1/100 of a unit by a single intradermal injection directly over the crop gland, confirming Lyons and Page.—M. H. F.

Suppression of persisting corpora lutea in hypophysectomized rats. Bunder, C. A., and R. O. Greep, Proc. Soc. Exper. Biol. & Med. **35:** 235. 1936.

Large doses of anterior pituitary extract were injected into female rats to produce heavily luteinized ovaries, after which the animals were hypophysectomized. Then, the effect of various hormonal preparations on the persisting corpora lutea was determined by daily injections over a 10-day period. Neither estrin nor progestin gave any evidence of influence on the persisting corpora, nor did the follicle-stimulating fraction of the A.P. Injection of luteinizing fraction, however, led to a marked diminution of ovarian weight, and pronounced involution of luteal tissue.—M. H. F.

Cyclic morphologic variations in the anterior hypophysis of the guinea pig. Chadwick, C. S., Am. J. Anat. **60:** 129. 1936.

The anterior hypophyses of 88 normal, sexually mature, female guinea pigs, killed at definite known periods of the estrous cycle, were studied both quantitatively and qualitatively. Changes in the hypophyses were correlated with changes occurring in the reproductive organs. It was found that in the lutein phase, when there were well-organized and active corpora lutea in the ovaries, together with many growing and differentiating follicles, both chromophile types of the hypophysis were reduced in relative percentage. In the follicular phase, when the corpora lutea were undergoing degeneration and all follicles developed in the current cycle were undergoing atresia except the relatively few destined to ovulate, the chromophiles were high in relative percentage and filled with granules. The basophiles were most numerous and fullest of granules in proestrus, but underwent degranulation and reduction in relative percentage in estrus. The eosinophiles were most numerous and fullest of granules in early estrus with some degranulation in late estrus and more extensive degranulation and reduction in relative percentage in metestrus. Both cell types remained at a low level during the subsequent lutein phase, rising again with the beginning of the next follicular phase.—Authors' Abst., Wistar Bib. Service.

The effects of glucose, fructose and galactose on ketosis produced by anterior pituitary extract and by pancreatectomy. Clark, D. E., and J. R. Murlin, J. Nutrition **12:** 469. 1936.

Normal dogs fed for several weeks on cream, then injected with an extract of anterior pituitary of beef prepared by the method of Magistrus developed severe ketosis. On control days basal levels of ketonemia, ketonuria, blood sugar, urinary nitrogen and R.Q.'s were determined at 8:00 A.M. and 12 NOON. Ten cubic centimeters of extract were then administered subcutaneously and the same determinations repeated at 4:00 P.M., 8:00 P.M. and 8:00 A.M. Blood and urinary ketones were greatly increased, likewise the blood sugar and urinary nitrogen. R.Q.'s were decreased. On adjacent days 50 gm. doses of the several sugars were given per os simultaneously with the extract. Invariably the ketosis and urinary nitrogen were diminished while blood sugar and R.Q.'s rose. Galactose was most effective, fructose next and glucose least in reducing ketosis in this type of experiment. Galactose also raised the R.Q. highest, but glucose sustained a high level longer. Nitrogen was spared most by galactose, followed in order by fructose and glucose. Depancreatized dogs also were fed the same sugars after withdrawal of insulin and establishment of typical diabetes. The same determinations were made. The three hexoses raised the blood sugar about equally. They also definitely re-

duced the ketosis and urinary nitrogen although the R.Q.'s remained at the diabetic level. Glucose was most effective on ketonemia, fructose least. Glucose and fructose spared nitrogen equally, galactose less.—Authors' Abst., Wistar Bib. Service.

Carbohydrate metabolism of hypophysectomized and hypophysoadrenalectomized rats. Corey, E. L., and S. W. Britton, Am. J. Physiol. **118:** 15. 1937.

Blood sugar, liver and muscle glycogen were determined on fully-fed rats (81 males and 17 females) at various intervals after hypophysectomy. Hypophysectomy was carried out by a modification of the method of Thompson and its success determined by (a) gross examination in the region of the sella; (b) testicular degeneration or ovarian atrophy and cessation of estrus; (c) cortico-adrenal atrophy. Hypophysoadrenalectomy was carried out in 12 rats and the carbohydrate metabolism followed. In the hypophysectomized rats the average sugar content of the blood rose for 30 to 40 days following operation to approximately 25% above normal. A subsequent fall to somewhat below the normal level occurred in long-surviving animals. The carbohydrate changes were apparently not correlated with nutritional or weight changes following hypophysectomy or with survival period. Observations by other workers on transitory polydipsia following ablation of the hypophysis were confirmed. Liver and muscle glycogen values in hypophysectomized (unfasted) rats varied widely, but were on the average, within normal limits. Adrenalectomy following hypophysectomy resulted in rapid and pronounced diminution in blood sugar and liver and muscle glycogen. Carbohydrate changes which have been observed in the chronic condition following hypophysectomy may be in part referable to effects on the adrenal cortex.—K. Brownell.

Vascular-renal effects of posterior pituitary extracts in pregnant women. Dieckmann, W. J., and H. L. Michel, Am. J. Obstet. & Gynec. **33:** 131. 1937.

The parenteral or intranasal administration of 0.5 to 2 minims of post-pituitary extracts produces decrease in the volume of urine, increase in the concentration of urinary chloride, and little or no rise in blood pressure of nonpregnant individuals (average 11 minims). Pituitrin given to patients with toxemia of pregnancy produced similar responses with but one exception, namely, a marked increase in the blood pressure (average 51 minims). This susceptibility was constantly noted in typical preeclamptic and eclamptic patients. The blood pressure either returned quickly to normal or remained increased for hours. Sixteen normal pregnant women, 12 patients with vascular-renal disease in pregnancy and 19 toxemic patients were studied. The rapid amelioration and occasional disappearance of all symptoms and signs within a period of two or three days in both preeclampsia and eclampsia indicate that if there was any disturbance of the pituitary gland, it was not organic.—E. L. S.

A new effect of prolactin on the ovary and uterus (Über eine neue Wirkung des Laktationshormons des Hypophysenvorderlappens auf Ovar und Uterus). Engelhart, E., Klin. Wchnschr. **15:** 424. 1936.

The daily administration of 3 to 4 pigeon units of prolactin to isolated rabbits induced marked luteinization of the stroma, which was associated with proliferative endometrial change and diminished pituitrin sensitivity of the isolated uterus; despite the fact that ovulation was not produced, and there was no intrafollicular luteinization.—M. H. F.

Quantitative investigations of anterior pituitary-like hormone in the urine (Quantitative Untersuchungen über die Ausscheidung von Hypophysen-vorderlappen-hormon im Harn). Ehrhardt, C., Klin. Wchnschr. **15:** 514. 1936.

The hormonal content of the urines of 30 pregnant women were investigated. In a perfectly normal pregnancy 70,000 M.U. of the hormone were found per liter of urine. In cases of vomiting of pregnancy 90,000 to 200,000 units per liter were found.—M. H. F.

A thyrotropic field surrounding the immature pituitary of the tadpole. Etkin, W., Proc. Soc. Exper. Biol. & Med. **34:** 508. 1936.

In tadpoles in which the pituitary primordia were removed and transplanted to a site near the developing thyroid, there was precocious development and markedly stimulated activity of the thyroid as judged by histological examination. In tadpoles in which there had been inserted heteroplastic thyroid primordia at site near the pituitary, there was some evidence of precocious development, and definite evidence of stimulation of the heteroplastic thyroid transplant, but not in the animals' own thyroid. From this the author concludes that around the pituitary of the tadpole there is a zone of thyrotropic effect.—M. H. F.

Concentration of gonadotropic substance from pregnancy urine. Fevold, H. L., and F. L. Hisaw, Proc. Soc. Exper. Biol. & Med. **34:** 712. 1936.

Cresol effectively adsorbed the gonadotropic activity from pregnancy urine, but not from castrate urine. Sufficient details of the method are given to enable its use for the routine recovery of P.U.—M. H. F.

Effect of hypophysectomy and replacement therapy on lactation in guinea pigs. Gomez, E. T., and C. W. Turner, Proc. Soc. Exper. Biol. & Med. **34:** 404. 1936.

It was impossible to restore or to maintain lactation with galactin in hypophysectomized lactating female guinea pigs or in hypophysectomized cryptorchid males bearing functional ovarian grafts. A suspension of whole sheep pituitary gland, however, was able to maintain lactation in 2 cases. These results are interpreted as indicating the importance of other endocrine glands in the process of lactation, i.e., thyroid and adrenal.—M. H. F.

Vacuolation of the anterior pituitary gland following castration, implantation of cancer tissue and thyroidectomy. Guyer, M. F., and Pearl E. Claus, Anat. Rec. **67:** 145. 1937.

Both cytological and experimental evidence is presented indicating that, in the hypertrophied basophiles of the anterior pituitary gland, the vacuolation which follows thyroidectomy is degenerative in nature, while that following castration is probably secretory and closely associated with the Golgi apparatus. Supplementary evidence from the liver cells of thyroidectomized and of castrated rats is presented. Rats bearing implanted carcinoma at first display vacuoles of the castrate type but the vacuolation of moribund animals may be more of the degenerative type.—Authors' Abst., Wistar Bib. Service.

Inhibitory effects by the hypophysis of rats following injections of pregnant mare serum hormone. Hamburger, C., Proc. Soc. Exper. Biol. & Med. **34:** 519. 1936.

Neither the sera of normal untreated adult female rats, of untreated castrate rats, nor of normal adult female rats injected with 50 M.U. daily of pregnant mare serum extract had any marked effect on the ovaries of mice when injected in 1 cc. quantities. However, after treatment with such amount of pregnant mare serum, the sera of spayed rats, hypophysectomized, and hypophysectomized and spayed rats, produced definite ovarian stimulation in mice upon injection in 1 cc. quantities. The injection of estrin together with the pregnant mare serum prevented the development of gonadotropic activity of the sera in castrated animals, but not in hypophysectomized spayed females. It is concluded that the hypophysis, under the influence of estrin, inhibits the action of gonadotropic hormone.—M. H. F.

Giantism: Report of case. Humberd, C. D., J.A.M.A. **108:** 544. 1937.

Of 124 letters and cards requesting reprints of and/or making comments about a recent paper of his on giantism, 71 informed the author of a remarkable schoolboy giant at Alton, Ill. This giant has received much newspaper and magazine publicity; the author's clippings about and photographs of him, over a period of 8 years, fill 2 large scrapbooks. But the popular accounts of his height vary so much in stating his dimensions that their accuracy was questionable. He recorded the history and measurements at his home, June 2, 1936: The giant is a white youth, single, aged 18 years 3⅓ months, is just completing the first semester of his freshman year in college. He is a preacromegalic giant, 8 feet 3¼ inches (252 cm.) tall, of phenomenal size, molded on a vast scale, colossal and stupendous in bulk, truly Gargantuan in all his proportions, and symmetrically built. His anthropometric picture is given. His present weight, he says, is 395 pounds (179 kg.) in ordinary clothes. The thick lips, coarse nose, increasing depth of the chin and general enlargement of the soft parts of the face, as noted when earlier photographs are compared, suggest that an acromegalizing process is already beginning and can be expected to become prominent in the near future.—Abst., A. M. A. Service.

Acromegaly and arterial hypertension (Acromégalie et tension artérielle). Jéquier, M., Ann. d'anat. path. **13:** 179. 1936.

In the case of a woman, 34 years old, who died of cerebral hemorrhage due to hypertension, the hypophysis contained a large acidophilic tumor which had eroded the sella and invaded the sphenoid sinus and compressed the rest of the hypophysis into a thin crescent. Other endocrine glands were apparently normal. Of 227 cases of acromegalia reported in the litera-

ture, 30 had increased blood pressure; 2, lowered pressure; 48, normal blood pressure.—A. T. R.

The role of the hypophysis in the development of high blood pressure, especially, essential hypertension (Die Bedeutung der Hypophyse für die Entstehung des Hochdruckes insbesondere der essentiellen Hypertonie). Jores, A., Klin. Wchnschr. **15:** 841. 1936.

By the use of a protein-free filtrate of 30 cc. of venous blood, it was possible to demonstrate the presence of a cortico-tropic principle by injection into mice. This principle increased the weight of the adrenals of the injected animals and altered the histological appearance of the glands in a manner similar to that of the corticotropic preparation from the anterior pituitary. Also its ultrafilterability, thermostability, resistance to proteolytic digestion, and its ether solubility, are identical with the corresponding properies of the A.P. corticotropic preparation. This principle was found in each of 6 cases of Cushing's disease, and in most cases of severe hypertension. It was not found in eclampsia or pre-eclampsia. In addition to the corticotropic principle, the author reports the presence of a principle which acts on the adrenal medulla, and which resembles the adrenalotropic activity of the A.P.L. From such experimental data the author concludes that the pituitary is intimately involved in the genesis of essential hypertension, and in the hypertension of Cushing's disease, but not in the hypertensive states of pregnancy.—M. H. F.

Effects of anatomical separation of hypophysis from hypothalamus in the dog. Keller, A. D., W. Noble and J. W. Hamilton, Jr., Proc. Soc. Exper. Biol. & Med. **34:** 794. 1936.

Section of the pituitary stalk did not uniformly produce diabetes insipidus in dogs as has been reported in cats.—M. H. F.

The influence of pregnancy urine and prolan upon the guinea pig thymus (Über den Einfluss von Schwangerenharn und Prolan auf die Meerschweinchenthymus). Klein, Fr., Klin. Wchnschr. **15:** 371. 1936.

The injection of pregnancy urine, or of prolan in amounts totaling 1000 to 2800 R.U., caused an atrophy of the thymus in a few guinea pigs (weight decrease, and histological change). In the female, this atrophy was accompanied by an apparent increase in the number of Hassall corpuscles.—M. H. F.

Preparation and assay of mammotropic hormone. Lyons, W. R., Proc. Soc. Exper. Biol. & Med. **35:** 645. 1937.

Details are given of a method for the preparation of a highly potent mammotropic pituitary fraction, active in 0.1 mg. in squabs when tested by the usual method, and in 0.001 mg. when tested by the intradermal local reaction. The preparation involves an acid-acetone extraction of the glands with subsequent iso-electric precipitation at pH 5.5. The product contains relatively little adrenotropic activity and no gonadotropic or thyrotropic activity when tested in 100 mg. doses in squabs, rats and guinea pigs. A similar extract prepared by iso-electric precipitation at pH 6.5 contains much adrenotropic and little mammotropic activity.—M. H. F.

Pituitrin injections and the blood picture in the normal and hypophysectomized guinea-pig. McFarlane, W. D., and M. K. McPhail, Am. J. M. Sc. **193:** 385. 1937.

Hypophysectomy of guinea-pigs did not result in any marked change in the red-cell and hemoglobin content of the blood. The injection of pituitrin in dosage of 5 to 10 cc. per kg. into normal and hypophysectomized guinea-pigs was followed after a 'rather prolonged' period by the development of a severe anemia. The postmortem examination of these guinea-pigs, some of which were still markedly anemic, did not show any occurrence of acute gastric lesions as have been reported by others. Microscopic examination of sections of the fundus failed to reveal any indications that ulceration had occurred.—R. G. H.

The pituitary gland and its relations to age, hypertension and pathological processes: a study of 107 unselected pituitaries. Parsons, R. J., Medical Papers Dedicated to Dr. Henry A. Christian, 366, Feb. 1936.

The individual cases are tabulated including the autopsy findings. Sample photomicrographs and numerous correlations tables are presented. The usual positive correlation between the number of basophilic cells in the posterior lobe and age is supported as is also the observa-

tion that this basophilic invasion is more conspicuous in men than in women. There was actually less invasion in females with hypertension than in those without high blood pressure; but in males the reverse was found. The author finally concludes that no significant correlation between invasion of the posterior lobe by basophilic cells and systolic or diastolic blood pressure is evident. Nodular hyperplasia was found in 19 of 107 glands. Five of these nodules (adenomas?) were composed of basophilic cells. Two of the basophilic nodules were in patients with hypertension and 3 were in patients without hypertension. The series included 1 case diagnosed as pituitary basophilism but the significant pathology was an adrenal cortical adenoma, the hypophysis not being definitely abnormal.—A. T. R.

Relation of anterior and posterior lobe of the hypophysis to insulin sensitivity in the rat. Pencharz, R. I., C. F. Cori and Jane A. Russell, Proc. Soc. Exper. Biol. & Med. 35: 32. 1936.

Removal of the anterior lobe of the pituitary in female rats led to a marked increase in insulin sensitivity as measured by the incidence of convulsions after administration of small amounts of insulin. The removal of the posterior lobe with little injury to the anterior lobe did not alter the normal sensitivity.—M. H. F.

Hypophyseal gonadotropic hormones and the luteinization phenomenon in the rat. Pfeiffer, C. A., Anat. Rec. 67: 159. 1937.

An investigation was made on the effects of copper sulfate upon the endogenous gonadotropic hormones, the evidence for 2 distinct hormones, and the distribution of the action of endogenous gonadotropic hormones. However, the effects of injected luteinizer are augmented when combined with copper salts. Injection of copper sulfate did not produce luteinization in the ovaries of 'constant estrus' females (with masculinized hypophyses) or ovarian grafts in the male, nor did it interfere with luteinization in the normal female. Implantation of the hypophyses from 5 castrate males bearing ovarian grafts caused the luteinization of the ovarian graft in the castrate male. However, 5 hypophyses of normal males bearing ovarian grafts failed to luteinize the ovarian graft in the normal male. This indicates that the physiological difference between the male and the female hypophyses is in the amount of luteinizing hormones that they are able to release. Since the results obtained in this investigation can be explained on the basis of a single gonadotropic hormone, a discussion of the gonadotropic hormones and their action in both sexes is included.—Authors' Abst., Wistar Bib. Service.

Copper hematoxylin, a stain for the acidophils of the human hypophysis. Rasmussen, A. T., Proc. Soc. Exper. Biol. & Med. 34: 760. 1936.

After sections had been stained by copper hemotoxylin, the subsequent counterstaining with acidophilic stains did not stain any cells not previously stained by the former reagent. Furthermore, decolorization of acidophils under the microscope and subsequent staining with copper hemotoxylin demonstrated that the latter is a stain for acidophils. These observations confirm those of earlier workers. The author cites these data as caution in the misinterpretation of tumors of the pituitary in cases of Cushing's syndrome.—M. H. F.

Influence of estrone upon galactin content of male rat pituitaries. Reece, R. P., and C. W. Turner, Proc. Soc. Exper. Biol. & Med. 34: 402. 1936.

The daily injection of 500 I.U. of estrone benzoate into male rats increased the galactin content as measured by the response of the pigeon crop gland to local intradermal injections.—M. H. F.

The effect of the growth hormone upon the brain and brain weight-body weight relations. Rubinstein, H. S., J. Comp. Neurol. 64: 469. 1936.

To determine whether cases of experimental gigantism would maintain the same brain weight-body weight relations as observed in normally large individuals, 74 white rats divided into one test and two control groups were studied. Group 1 received intraperitoneal injections of growth hormone; group 2, intraperitoneal injections of meat extract; group 3 remained uninjected. After 22 weeks of injections the animals were sacrificed and studied. The growth hormone produced generalized body growth. It failed to influence the structure of the central nervous system as judged by studies of weight, volume, density, water and solid contents of the brain. It affects the normal brain weight-body weight relations by decreasing the brain weight-body weight ratio, by lowering the exponent of relation and by leading to a

significant deviation from the body-brain growth curves as established for the normal. It increases the size of the male pituitary gland but fails to affect the normally large pituitary gland of the female. Hence, it tends to restore the size of the pituitary gland of the white rat to that normal for the Norway rat.—Authors' Abst., Wistar Bib. Service.

PANCREAS

Experimental studies on the effects of insulin, protamine insulin, and crystalline insulin. Barach, J. H., Pennsylvania M. J. **40:** 349. 1937.

Since it has been determined that insulin can be modified so that absorption is delayed, 4 new possibilities have arisen. The first is a more gradual blood sugar-lowering effect; the second is a continued effect over a longer period of time. Together these factors have produced a highly desirable result—an increase in the total amount of blood sugar reduction, which means greater glucose utilization per unit of insulin over a given period of time. In the author's studies crystalline insulin showed this effect more often than standard or protamine insulin, having accomplished this result in more than half of the cases. The third advantage is that of reducing the incidence of insulin reactions and insulin shock, and the fourth is in the reduction of the number of doses of insulin per day. Crystalline insulin offers all of the advantages of the present-day modifications of standard insulin; thus far the author has encountered none of the disadvantages.—I. B.

The frequency and characteristics of insulin-sensitive and insulin-resistant diabetes (Über Häufigkeit und Besonderheiten des Insulinempfindlichen und Insulinresistenten Diabetes). Fenz, E., Klin. Wchnschr. **15:** 681. 1936.

Over a five-year period 362 diabetics were investigated and classified into insulin-sensitive cases, characterized by high glucose-to-insulin ratio, marked inclination to ketosis, high blood cholesterol which is quickly lowered by insulin, and quick reappearance of marked metabolic disturbance on insulin withdrawal; and, insulin-resistant cases, with low glucose/insulin ratio, only moderately elevated blood cholesterol which does not respond to insulin, and little inclination to ketosis. Of the 362 cases investigated, 51.4% were of the insulin-resistant group (i.r.) and 34.2% of the insulin-sensitive group (i.s.). The remaining 14.4% were of a middle group. The i.r. group proved to be at or past middle age, and evidenced a high incidence of obesity, elevated blood pressure, and complications. The i.s. group was composed of younger individuals, relatively free from adiposity and high blood pressure. The author concludes that insulin resistance, adiposity and high blood pressure are constitutional factors which frequently occur together, but which do possess a certain amount of independence.—M. H. F.

Diabetic arteriosclerosis. Henderson, T. A., Pennsylvania M. J. **40:** 329. 1937.

Data pertaining to 8 patients are thus summarized: The average age exclusive of the 1 nondiabetic patient was 62. All diabetic cases were mild. Three were controlled on no insulin, 1 on 5 U., 1 on 15 U., 1 on 40 U., and 1 on 50 U. Of the 8 patients, 5 had amputations because of infection and only 3 because of uncomplicated ischemia or true gangrene. The order of involvement of the arteries seemed to be posterior tibial, peroneal, and anterior tibial. The extent of the arterial changes corresponds much more to the duration of the diabetes than it does to the age of the patient. It is realized that observations on such a small group of cases are of little value. It is realized also that, since all of these patients except one were over 50, they would be expected to have some changes in the arteries even if they did not have diabetes.—I. B.

On the effect of cholesterol and insulin on yeast fermentation (Über die beeinflussung der Hefegärung durch Cholesterin and Insulin). Hermann, L., and R. Neiger, Biochem. Ztschr. **281:** 121. 1935.

Cholesterol solution decreased considerably the fermentative activity of yeast, but activated yeast autolysates. This is probably not a colloidal phenomenon because neither mastix nor paraffin solutions show this effect. By petroleum ether extraction yeast was sensitized against salicylic acid and sulfuric acid poisoning. The stimulating effect of insulin preparations (norgine and organon) on yeast fermentation was considerably decreased by cholesterol. Fermentation of yeast autolysates was not stimulated by insulin. It is questioned that cozymase is the stimulating factor in insulin.—F. Breuer.

Diabetes today and tomorrow. Joslin, E. P., Ann. Int. Med. **10:** 179. 1936.

A commentary with especial emphasis on occurrence, duration and hereditary tendency of diabetes and comparison of old and new methods of treatment. The author describes and emphasizes the value of new methods of instruction and care of the diabetic.—R. R. Durant.

Diabetes. Joslin, E. P., Pennsylvania M. J. **40:** 325. 1937.

With protamine insulin the fundamentals of treatment of diabetes mellitus are more nearly achieved. Diabetes today is a disease to be respected, and neglect to do so spells diaster. Diet and exercise are as essential as ever. The patient must not overeat, but it seems likely that after the insulin dosage for a given quantity of carbohydrate has been determined, the protein may be determined by adjustment to the age and size of the patient, fat may be regulated by body weight, and even the carbohydrate may vary 10 to 20% up or down with comparative impunity. The simplicity of the administration of insulin in 1 or 2 doses instead of 2, 3, or 4 doses will appeal so generally to patients that the probability is strong that the number of diabetics taking insulin will increase. As a result the percentage of deaths from coma should fall at an accelerated pace. Best of all is the hint that the more complete control of the disease which the new insulin makes possible may so raise the standard of bodily health that the diabetic will be less subject to, and will resist more successfully, the various so-called diabetic complications—infections, vascular degeneration, and abnormal neurologic and ophthalmologic manifestations.—I. B.

Factors influencing the stability of insulin. Sahyun, M., M. Goodell and A. Nixon, J. Biol. Chem. **117:** 685. 1937.

Insulin low in ash and free from copper, iron, and zinc was prepared by repeated precipitation at the iso-electric point and by alcohol-ether mixtures. This material lost 50% of its activity when kept at 52° C. for 9 weeks. The deterioration was less marked when 1 mg. of copper or 1 mg. of iron was added to each 1000 U. of insulin. The addition of 1 mg. of zinc per 1000 U. of insulin decreased the loss of activity at the end of 9 weeks to 10%, and there was no change in the time of onset or duration of the hypoglycemic effect.—J. M. L.

An insulin-sparing action of copper (Über eine insulinsparende Wirkung des Kupfers). Schnetz, H., Klin. Wchnschr. **15:** 646. 1936.

Earlier publications are cited in which it was reported that copper, zinc, and cadmium, inhibited the adrenalin glycogenolysis of the isolated frog liver, and the adrenalin hyperglycemia of rabbits. In the present experiments it was demonstrated that neither one dose of copper sulphate of 15 mg., nor repeated doses totaling 20 mg., had any effect on the fasting blood sugar of normal men. However, after the administration of 20 mg. of the salt daily for 2 to 3 days, the adrenalin hyperglycemia was diminished (0.5 cc. adrenalin subcutaneously). Similarly, the administration of copper diminished the alimentary hyperglycemia in normal patients. In 2 diabetic patients the oral administration of copper decreased the hyperglycemia and glycosuria, and decreased the amount of insulin needed.—M. H. F.

Pancreatic diabetes and hypophysectomy in the rat. Shapiro, R., and G. Pincus, Proc. Soc. Exper. Biol. & Med. **34:** 416. 1936.

Removal of the greater part of the rat pancreas produced a prompt rise in blood sugar which was maintained at a steady high level. Hypophysectomy restored the blood sugar to normal. Hypophysectomy without pancreatectomy resulted in hypoglycemia unless additional sugar was given.—M. H. F.

PARATHYROIDS

Parathyroid hyperplasia in rabbits produced by parenteral phosphate administration. Drake, T. G., F. Albright and B. Castleman, J. Clin. Investigation **16:** 203. 1937.

The parathyroid glands of rabbits given intravenous injections of phosphate showed histological hyperplasia. The dose was 75 mg. P daily, at pH 7.3. The 19 control animals had glands with average weight 13 mg., the treated animals averaged 20 mg. The results support the hypothesis that in chronic renal insufficiency the hyperphosphatemia leads to parathyroid hyperplasia.—E. L. S.

Effect of calcium and parathormone on serum calcium in normal, Eck fistula and gastrectomized dogs. Lederer, L. G., and L. A. Crandall, Jr., Am. J. Physiol. **118:** 52. 1937.

Fasting serum calcium of the Eck fistula dog is significantly below that of the normal animal. Other results which show that the calcium metabolism in Eck fistula dog is not normal, are: 1) a decreased rise in serum calcium after feeding calcium glyconate and calcium lactate by mouth, 2) a more rapid loss of calcium from the blood after intravenous injection of $CaCl_2$ and 3) a decreased effect of parathormone given subsutaneously—7 units of parathormone per kg. given to Eck fistula dogs caused only 70% as much rise as it did in normal dogs. Disturbances in liver function, particularly a deficiency in the secretion of bile may lead to inadequate absorption of calcium in digestive tract. The absence of gastric secretion does not seriously interfere with the absorption of large doses of calcium. Ingestion of calcium lactate by gastrectomized dogs causes much larger increase in serum calcium than does calcium gluconate. The difference is not explained.—S. R. Tipton.

PINEAL

The hormonal effect of the pineal (Zur Frage der hormonalen Wirkung der Zirbeldrüse). Fleischmann, W., and H. Goldhammer) Klin. Wchnschr. **15:** 1047. 1936.

The implantation of pineal glands of immature animals into adult female rodents, inhibited the estrous cycle, but such implantation was incapable of antagonizing the usual effects of the injections of gonadotropic extracts into immature rats or rabbits. The pineal glands of mature animals did not possess this property.—M. H. F.

THYROID

Galactose tolerance in hyperthyroidism. Althausen, T. L., and G. K. Wever, J. Clin. Investigation **16:** 257. 1937.

The galactose tolerance test was with 40 gm. orally, blood samples analyzed at 5, 15, and 30 minutes. It appeared that the curve is higher in thyrotoxics than with normals or diabetics. (The latter group are normal to this test.) Thyroidectomy usually returns the galactose curve to normal range. The results appear to be uniform enough to be diagnostically helpful. The data are from 26 patients compared with 35 others.—E. L. S.

Effect of different forms of iodine on laying hens. Asmundson, V. S., H. J. Almquist and A. A. Klose, J. Nutrition **12:** 1. 1936.

In a series of experiments White Leghorn hens were supplied with iodine from oyster shell, desiccated thyroid, iodo-salicylic acid, sodium iodide, potassium iodide, potassium iodate, di-iodotyrosine and iodized olive oil. Samples of egg, excreta and some of the tissues were analyzed for iodine. Body weight, egg production and feed consumption was not adversely affected except in the case of birds given desiccated thyroid and high levels of sodium iodide. The iodine content of eggs corresponded only approximately to iodine intake and differed according to the source of iodine. Thus eggs laid by birds given iodo-salicylic acid had a comparatively low iodine content, while eggs from birds fed potassium iodide, potassium iodate and di-iodotyrosine had a significantly higher iodine content. Eggs laid by hens given sodium iodide were apparently comparable to those from hens fed potassium iodide while eggs from hens given other iodine supplements were intermediate. A higher percentage of the total iodine intake was excreted in feces of birds given iodo-salicylic acid than in the case of birds given potassium iodide or di-iodotyrosine. Iodine was found in the thymus and other tissues beside the thyroid but none of the differences between groups of birds were significant. These results indicate that iodine utilization by laying hens, in so far as it can be measured by the iodine content of eggs and the excreta, depends upon the source as well as the amount of iodine given.—Authors' Abst., Wistar Bib. Service.

The behavior of the antithyroidal substance of the blood of normal people under the influence of diiodotyrosine (Das Verhalten der antithyreoidalen Schutzstoffe des Blutes Gesunder unter Dijodtyrosinwirkung). Bodart, F., and K. Fellinger, Klin. Wchnschr. **15:** 1005. 1936.

From the serum of normal individuals, an ether-soluble extract representing 150 cc. of blood inhibited the thyrotropic manifestations of an injected A.P. extract when the former was fed to guinea pigs. Less of this antagonist was found in hyperthyroidism; more in myxedema. Although the administration of diiodotyrosin to thyrotoxic patients led to an increase of the antagonist in the patient's blood, this substance caused no change in the blood level of the antagonist when administered to normal patients.—M. H. F.

The effect of thyroidectomy on blood lipids. Boyd, E. M., Trans. Roy. Soc. Canada **30:** 11. 1936.

In 10 patients, sub-total thyroidectomy produced a mild plasma lipemia but had no effect on the lipids of red blood cells. While the increased plasma lipid values were within the normal range, they were considered to represent a moderate lipemia when compared with the pre-operative level. The approximate increase, 2 or 3 weeks post-operative, was 100% in neutral fat, 30% in free cholesterol, 20% in ester cholesterol and 10% in phospholipid. No further figures are given but a typical case is illustrated graphically. The author's modification of Bloor's oxidative methods were used.—Lowell O. Randall.

Therapeutic hyperthyroidism. Bram, I., Internat. Clin. **1:** 48. 1937.

One hundred and three patients were subjected to therapeutic hyperthyroidism. Of these, 24 presented simple colloid goiter; 22, obesity with hypothyroidism; 15, obesity without hypothyroidism; 13, hypothyroidism without obesity; 14, myxedema, and 15 presented dystrophia adiposa genitalis (Froehlich's disease). The results of treatment were as follows: 6, or 5.8%, were not improved; 17, or 16.5%, were partially improved; 28, or 27.2%, reached the point of near recovery, and 52, or 50.5%, achieved recovery. By the term therapeutic hyperthyroidism as employed in this contribution is meant a state of mild thyrotoxicosis induced by the administration of gradually increasing doses of thyroid substance to the point of an increase to within 10 points beyond the patient's maximal normal basal metabolic rate and an increase of the heart rate to within the maximum of 90 per minute. The indications for therapeutic hyperthyroidism appear to be such clinical manifestations of hypometabolism (the basal metabolic rate in occasional instances notwithstanding) as are of protracted duration and respond tardily to the conventional mode of thyroid administration. It would appear that therapeutic hyperthyroidism, cautiously applied, deserves serious consideration in selected cases of simple colloid goiter, obesity, myxedema, and dystrophia adiposa genitalis.—Author's Abst.

Thyroid disturbances and chloremia (Troubles thyroïdiens et chlorémie). Decourt, J., and C. Guillaumin, Compt. rend. Soc. de biol. **121:** 1332. 1936.

Chloride determinations were made on the blood of 5 human subjects with hyperthyroidism and of 3 with myxedema, before and after clinical treatment. Similar determinations were made on the blood of 3 rabbits injected with thyroid extract. It was found that clinically and experimentally hyperthyroidism was associated with an increase in blood cell and blood plasma chlorides. In myxedema the chloride content of the blood was low.—E. Cutuly.

Inhibition of the effect of thyroxin on blood cholesterol by anesthesia of the diencephalon (Blockierung der cholesterinsenkenden Wirkung von Thyroxin und thyreotropen Hormon durch Zwischenhirnnarkose). Fenz, E., and F. Zell, Klin. Wchnschr. **15:** 1133. 1936.

From acute experiments on rabbits lasting no more than 4 hours, the authors conclude that veronal or chloralose inhibits the usual effect of thyroxin or thyrotropic hormone on blood cholesterol.—M. H. F.

Modifications in the thyroid gland of rabbits following superior cervical ganglionectomy (Modifications de la glande thyroïde consécutives à la ganglictomie cervicale supérieure chez le lapin). Florentin, P., T. Fontaine and L. Hennequin, Compt. rend. Soc. de biol. **121:** 1410. 1936.

Presented in this report are the histological changes seen in the thyroid glands removed at various intervals from a few hours to 3 weeks following bilateral cervical ganglionectomy in rabbits. Shortly after the operation the follicles of the thyroid increased in size and contained fluid colloid whose periphery was vacuolated; the acinar epithelium was tall. Subsequent to this phase of hypersecretion and rapid resorption of colloid, the epithelium of the follicles became lower, peripheral vacuolation of the colloid disappeared, and the follicles became markedly distended with hard colloid. Control unilateral ganglionectomy did not affect the structure of the lobe of the thyroid on the side operated. Hyper- and hypo-activity of the thyroid is said to parallel that occurring in the hypophysis.—E. Cutuly.

Comparison of effects of various thyroid preparations on rats. Freud, J., and E. Laqueur, Nederl. tijdschr. v. geneesk. **80:** 25. 1936.

Thyroid preparations were compared with reference to their effect upon basal metabolism of rats according to the method of Abelin. One half of the animals got in the first test one of

the preparations with which in the second experiment the other group of rats was treated. It appeared that thyroid preparations are active in ratio with their total iodine content. Parenteral administration proved to be more effective than oral. Preparations which may be used for parenteral administration, such as injectable thyranon (prepared by peptic and tryptic digestions) or elityran (containing the thyroglobulin fraction) are also active in ratio with their total iodine content.—L. Meyler.

Vitamin C and thyroid in their relation to bone regeneration (Vitamin C und Schilddrüse in ihren Beziehungen zur Knochenregeneration). Hanke, H., Klin. Wchnschr. **15:** 1121. 1936.

In guinea pigs, experimentally-produced holes in long bones did not heal unless vitamin C was present in the diet. In rabbits, however, healing occurred in the absence of vitamin C from the diet. Yet, in both species, the addition of vitamin C accelerated bone repair. The total removal of the thyroid did not prevent the bone repair, although the administration of thyroid to normal animals did accelerate the process.—M. H. F.

The cytology of snake thyroids following hypophysectomy, activation and ultracentrifuging. Hellbaum, H. W., Anat. Rec. **67:** 53. 1936.

The thyroids of 334 snakes were used in this study. Normal snake thyroids, are similar to those of other animals in that they have low columnar to cuboidal type of epithelium, apically placed Golgi network, and basally located nuclei. After 6 daily injections of anterior pituitary the cells become highly columnar and the Golgi apparatus enlarges into a prominent network. Langendorff cells are more numerous, and the follicles are relatively smaller in size than those of the controls. One series of 46 hypophysectomized snakes autopsied at various times showed regressive changes in that the epithelium became more flattened, the Golgi material more pycnotic, and the follicles enlarged. Another series of 57 snakes hypophysectomized for 30 days was injected daily with anterior pituitary. After 4 or 5 days of such replacement therapy, the epithelium was brought back to normal, and after 10 days, showed extreme exaggeration. Longer periods of injections changed the histological picture by increasing the number of Langendorff cells, and by causing the epithelium to almost obliterate the lumen. After 6 days of activation the thyroids were ultracentrifuged for 20 and 60 minutes. Stratification showed the order of specific gravities from highest to lowest to be as follows: nucleus, cytoplasm with colloid vacuoles, denser cytoplasm and Golgi apparatus.—Author's Abst., Wistar Bib. Service.

Effect of hyperthyroidism on sympathetic nervous system. Hepler, Opal E., and J. P. Simonds, Proc. Soc. Exper. Biol. & Med. **34:** 534. 1936.

The injection of adrenalin under ether anesthesia was no more effective in hyperthyroid animals than in normal dogs, and construction of the hepatic veins produced a fall in blood pressure more marked in hyperthyroid than in normal dogs.—M. H. F.

Clinical study of atypical blood iodin values (Zur Klinik atypischer Blutojodwerte). Kisch, F., Klin. Wchnschr. **15:** 342. 1936.

Employing the method of Leipert, the extremes of normal blood iodin values were found to be 8 and 17 micromilligrams. In every case of abnormally high-blood iodin there was found an increased metabolic rate although some cases of abnormally low-blood iodins were found without a lowered B.M.R. In many cases an increased B.M.R. was not accompanied by a raised blood iodin. These cases are interpreted as being instances of increased metabolic activity not due to hyperthyroidism, which presumably would not react to the therapeutic test of iodin administration. The author advances the thesis that hyperthyroidism does not exist without an increased blood iodin and that the latter is always accompanied by an increased B.M.R. Since, however, an increased B.M.R. is not always accompanied by an increased blood iodin, it is assumed that the regulation of total metabolism and iodin metabolism are not necessarily directed from identical points.—M. H. F.

A study of the thyroid in embryos of Eleutherodactylus nubicola. Lynn, W. G., Anat. Rec. **64:** 525. 1936.

The Jamaican tree toad possesses no aquatic larval stage but lays terrestrial eggs from which the young hatch as fully metamorphosed individuals. During embryonic development fore and hind legs appear at about the same time and develop at nearly equal rates; there are no gills; the broad, heavily vascularized tail disappears shortly after hatching. An investigation

of the thyroid was undertaken to ascertain the time of appearance of the gland and the time of first discharge of its colloid and to correlate these with the progress of bodily differentiation in the developing embryo. At a stage when the limbs have already appeared as buds, sections show no indication of the presence of the thyroid. It is not until the toes are clearly differentiated that the primary follicles are organized and actively secreting. At later stages the follicles increase uniformly in size and at the time of hatching fusion of primary follicles has occurred. The changes in staining reaction of the colloid follow their usual course, the droplets in the primary follicles staining uniformly blue with Mallory's connective tissue stain but later showing red centers and blue borders. From the material available it appears that, though active secretion and storage of colloid occurs during embryonic life, there is no striking period of discharge of the secretion analogous to what occurs at metamorphosis of ordinary tadpoles.—Author's Abst., Wistar Bib. Service.

The effect of small doses of potassium iodide on the thyroid gland of the guinea pig. Margolin, E. S., J. Pharmacol. & Exper. Therap. **59:** 277. 1937.

Previous investigations have shown that potassium iodide, in doses of 0.01, 0.05 or 0.1 gm. daily, stimulated the thyroid gland of young adult guinea pigs. In this series, 0.1 or 0.5 mg. of potassium iodide was fed daily to male guinea pigs weighing about 200 gm. each. The thyroid glands were examined after feeding periods of 5, 10, 15 and 20 days. The average number of mitoses in the thyroid glands of a series of 8 normal guinea pigs, counting every tenth section of one lobe, was 171. The average number of mitoses in the thyroids of the guinea pigs receiving potassium iodide was 571. Other morphological characteristics of the thyroid glands of guinea pigs receiving potassium iodide also indicate stimulation. The stimulating effect seems to reach a maximum in 15 days.—E. L.

Resistance of thyroparathyroidectomized rats to growth of a spindle-cell sarcoma. McJunkin, F. A., R. D. Templeton and F. G. Kravec, Proc. Soc. Exper. Biol. & Med. **34:** 801. 1936.

In over 1000 control animals the transplanted sarcoma has not yet regressed in a single instance. In thyroparathyroidectomized animals regression of the tumor occurred in one-third of the animals. If only the animals operated on at a suitably long interval are considered, the number of regressions amounted to over 70%.—M. H. F.

Total thyroidectomy for intractable cardiac decompensation. Minnich, W. R., and D. H. Poer, Piedmont Hospital Bulletin **10:** 2. 1937.

A case is reported in which the therapeutic results were strikingly satisfactory.—R. G. H.

Influence of the thyroid on vitamin C reserves (Influence de la thyroïde sur les réserves de vitamine C). Nespor, E., Compt. rend. Soc. de biol. **122:** 427. 1936.

The average vitamin C content of the adrenals of 4 rabbits treated with desiccated thyroid, as compared with that of 4 control animals, showed a decrease of 52%. In 7 guinea pigs treated with thyroid the average loss in vitamin C content was 59% in the adrenals and 48% in the spleen (9 animals were used as controls). To determine the influence of the thyroid on vitamin C retention in the organs of guinea pigs, 5 thyroidectomized animals, 6 animals treated with thyroid substance and 4 controls were subjected to identical treatment with ascorbic acid; aqueous solutions of the vitamin were administered orally. Determinations of retained vitamin C in these animals expressed as mg. per gm. of organ tissue gave rather inconstant results. Constant results were obtained if the vitamin C content was expressed as the ratio of the total organ content (mg.) and body weight (gm.). Thus, this ratio (average) in controls was 0.54, in thyroidectomized animals 0.59, and in thyroid-treated animals 0.43, indicating increased retention of vitamin C in the absence of thyroid, and decreased retention in the presence of excess thyroid.—E. Cutuly.

Further investigations on the site of action of thyroxin (Weitere Untersuchungen über den Ort der thyreogenen Stoffwechselwirkung). Oberdisse, K., and E. Roda, Klin. Wchnschr. **15:** 1094. 1936.

The oxygen consumption (Warburg technique) of denervated kidney from rabbits previously injected with thyroxin, was just as high above the normal values as the oxygen consumption of the contralateral kidney with intact nerve supply. The author correlates these findings with other pertinent findings in the literature and concludes that the action of thyroxin is directly on the tissue cells without any intermediation of the nervous system.—M. H. F.

On the question of the phosphatid metabolism and the influence of thyreoidin (Zur Frage des Phosphatidstoffwechsels und seiner Beeinflussung durch Thyreoidin). Pasternak, Lydia and I. H. Page, Biochem. Ztschr. **282:** 282. 1935.

The previous observation of the authors that thyroxin and thyreoidin increases the muscle phosphatids in mice by 96% cannot be explained as due to an inability to break down the phosphatids, because phosphatids, injected intraperitoneally are eliminated, not stored. Furthermore the total amount of phosphatids in the body also is increased (40%). The increase in the muscle, therefore, cannot be explained as a shift of material from other tissues. Also the content of cholesterin increases, both in the muscles and in the whole body. The results make it probable that phosphatids are formed under the influence of thyroxin, possibly as intermediary products of the fat metabolism.—E. Bozler.

The blood iodine level before and after subtotal thyroidectomy for hyperthyroidism. Perkin, H. J., and L. M. Hurxthal, New England J. Med. **215:** 698. 1936.

In a group of 141 patients with hyperthyroidism the blood iodine was determined preoperatively and at intervals of 3 months after operation. The group of 83 patients having an elevated blood iodine, averaging 26.0 micrograms %, showed a fall to normal levels within 3 months after the operation. The group of 58 patients with normal blood iodine, averaging 7.0 micrograms % before operation, showed an increase after operation amounting to 12.0 at 3 months and 21.5 at 6 months, and then a fall to normal levels. It was suggested that a more radical subtotal thyroidectomy should be performed in hyperthyroid cases with a normal blood iodine value to reduce the incidence of recurrence.—J. M. L.

Calcium and iodine metabolism in thyroid disease. Puppel, I. D., and G. M. Curtis. Arch. Int. Med. **58:** 957. 1936.

Two normal subjects on a low Ca diet showed a negative Ca balance. The Ca and P content of the serum of 1 totally thyroidectomized subject and 2 patients with exophthalmic goiter was normal but the latter showed augmented Ca excretion, and the iodine balance was less than normal even in the presence of normal intake. An increase in the excretion of Ca accompanied increase in the circulating iodine. With a low intake of Ca, observations during 3 periods of 3 days each revealed that the iodine balance was continuously negative and that the Ca balance varied directly with the iodine balance. With a high intake of Ca, observations during 5 periods of 3 days each showed that the iodine balance remained at a level definitely lower than that of the 2 normal controls. The Ca balance varied inversely with the iodine balance. In the patient with hypothyroidism a decrease in the excretion of Ca accompanied the decrease in the circulating iodine. In the other subjects maintained on a low Ca diet, observations during 9 of 12 periods of 3 days each showed the Ca balance to vary directly with the iodine balance.—I. B.

Hyperthyroidism masked by symptoms of acute abdominal catastrophe. Robertson, W. E., M. G. Wohl and H. F. Robertson, J.A.M.A. **108:** 623. 1937.

The authors consider instances in which the acute symptoms and signs referable to the abdomen are due entirely to the overactivity of the thyroid. The symptoms referable to the abdomen may be so severe as to suggest a surgical catastrophe. In 2 of 3 such cases the abdominal signs mimicked acute appendicitis. One of these patients was operated on and a normal appendix was found. The pains in the third patient were localized in the left upper quadrant of the abdomen. Postmortem examination showed no abdominal pathologic condition to account for the pains. Differentiation is not always possible, since hyperthyroid patients may harbor lesions of the gastro-intestinal tract. When appropriate therapy is applied, the abdominal symptoms disappear with other signs of hyperthyroidism. In the surgical cases the signs and symptoms usually progress.—Abst., A. M. A. Service.

The genesis of thyroid protein. Salter, W. T., and J. Lerman, New England J. Med. **216:** 371. 1937.

An artificial protein, which possessed marked metabolic activity in human myxedema, was synthesized by the enzymatic synthesis from inactive diiodotyrosine peptone with pepsin. The protein had many of the quantitative chemical and biological characteristics of natural protein. The effect of iodine therapy in hyperthyroidism was ascribed to a mass-law action which permitted the production and accumulation of diiodotyrosine and its subsequent synthesis into thyroid hormone in the gland.—J. M. L.

Experimental production of exophthalmos resembling that found in Graves' disease. Smelser, G. K., Proc. Soc. Exper. Biol. & Med. **35:** 128. 1936.

The daily injection of a large dose of thyrotropic anterior pituitary extract into guinea pigs produced marked exophthalmos in thyroidectomized animals but not in normal animals. This exophthalmos was shown by autopsy examination to be dependent largely upon fatty infiltration into the orbital tissues, and was not dependent upon an intact sympathetic innervation to the orbital structures.—M. H. F.

Oestrin in toxic goitre. Spence, A. W., Lancet **2:** 970. 1936.

In 6 women having toxic goitre, neither the administration of dehydroxyestrin, 50,000 I.U. daily for 12 to 21 days intramuscularly nor 50,000 units 5 times a day for 21 days orally, had any favorable influence. The theory that estrin has an anti-thyrotropic effect was not supported.—L. B. S.

Athyroidism in the salamander Triturus torosus, Rathke. Taylor, A., J. Exper. Zoöl. **73:** 153. 1936.

Adult salamanders in the athyroid condition tend to be more edematous, the specific gravity of the body becomes lower, less oxygen is used, and a thicker more leathery skin develops than normal. As the thyroidless state is prolonged the animals exhibit a progressive emaciation. A study of the histological effects of the thyroidless condition in the salamander revealed that the exocrin glands particularly the granular glands of the skin and the glands associated with the digestive tract are structurally disturbed. Abnormal morphological changes in these glands appear to arise before the animal is seriously affected otherwise. In advanced cases of the athyroid condition the state of the tissues indicate nutritional deficiency. This is seen especially in liver tissue, connective tissue, and in tissues such as that of the epidermis which are subject to rapid replacement. It is suggested that the metabolic changes which accompany thyroid disfunction may be in part mediated by disturbances to nutrition.—Author's Abst., Wistar Bib. Service.

The effect of iodin on the thyroid (Zur Frage der Schilddrüsenwirkung des Jods). Weil, R., Klin. Wchnschr. **15:** 387. 1936.

On autotransplanted guinea pig thyroids, either in the normal condition or after stimulation by thyrotropic extract, the administration of iodin either in the form of potassium iodid, di-iodotyrosin, or thyroxin, produced the usual regressive changes in the cells with the attendant storage of colloid. In both conditions of the gland, the effective doses of the potassium salt were accompanied by apparent cellular injury, although this was not apparent with thyroxin, and only perceptibly so with the di-iodotyrosin. The author therefore concludes that the action of the iodids is directly on the thyroid gland, and not indirectly through the pituitary, or the nervous system.—M. H. F.

Experimentally induced hyperthyroidism in the chick embryo. Woodside, G. L., Anat. Rec. **67:** 423. 1937.

Thyrotropic hormone (Collip) was injected into chick embryos ranging in age from the 10th to the 20th day of incubation, and a study made of the effects on the embryo. An opening was cut in the shell and outer shell membrane in the region of the air sac. A hypodermic needle was then inserted through the inner shell membrane and allantois, allowing the hormone to be injected directly into the embryo. Completely activated thyroids, containing high epithelia and showing an absence of colloid, were produced within 24 hours by direct injections into embryos ranging in age from 12 to 20 days of incubation. Equivalent or larger quantities of the hormone were ineffective when placed in the air sac for the same length of time or for longer periods. Prolonged direct injections of thyrotropic hormone resulted in involutional changes in the thyroids of embryos treated from the 11th to the 20th day of incubation. A lowering of the follicular epithelium and a reappearance of vacuolated colloid became marked on the twentieth days. Embryos receiving prolonged treatment showed consistent loss in weight, diminution of fat storage and a slight degree of emaciation as compared with treated and untreated controls.—Author's Abst., Wistar Bib. Service.

Antagonism between the effects of dessicated thyroid and unsaturated fatty acid (Zur antithyreoidalen Wirkung einiger ungesättigter Fettsäuren). Zain, H. Klin. Wchnschr. **15:** 1722. 1936.

The administration of small amounts of unsaturated fatty acids to female rats protected the liver glycogen from the usual effects of experimental thyrotoxicosis. Saturated fatty acids were without effect in equal doses. Neither type of acid gave any protection to male rats similarly treated.—M. H. F.